FAILURE OF FEATHER PIGMENTATION IN BRONZE POULTS DUE TO LYSINE DEFICIENCY

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ONE FIGURE

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Turkey growers who raise bronze poults in complete confinement are frequently disturbed by the appearance of an unusual extent of white coloring in the primary and secondary wing feathers. The tail feathers may also be affected. The abnormal pigmentation is most apparent during the first 4 or 5 weeks, and gradually disappears as the birds become older, even though the diet and conditions of management remain the same (Carrick, '45).

Funk and Kempster ('40) observed a white feather development in bronze poults when 10% cottonseed meal or 10% corn gluten meal was used in the ration. Feather pigmentation was normal when soybean meal was similarly used in the ration. They also recorded that the abnormality diminished as maturity approached, and they postulated that the pigmentation failure was due to "a deficiency of some constituent which was required for rapid growth but was released for pigmentation when the growth rate diminished." These authors also suggested that "manganesed calcium" used in some of the rations may have been a causative factor. Roberts ('45) noted that white plumage developed in bronze poults when 20% soybean meal in the ration was replaced with ground corn.

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A series of reports from the Oklahoma Experiment Station (summarized by Ewing, '43) indicated that low fiber rations caused a similar loss of pigment in bronze poults. However, it might be noted that the source of fiber used in these studies was alfalfa stem meal which may have contributed many factors in addition to fiber.

Excessive white feather formation has also been reported in growing chickens (McConachie, Graham, and Branion, '35; and Poley, '38) but in these reports it is not clear that the causative factor was the same as in the cases involving poults. Groody and Groody ('42) reported that pantothenic acid deficiency caused less feather pigmentation in chicks.

The object of this investigation was to study the possible causes of the loss of pigment from the feathers of poults, and to determine how this syndrome can be prevented.

EXPERIMENTAL

Standard bred bronze poults, hatched from the Borden Experimental Farm flock, were used in this study. The day-old poults were placed in electrically heated, wire floored, battery brooders. Throughout the test they received the designated diets and fresh tap water ad libitum. The first test was designed to study the effect of varying fiber and calcium content, and of the use of corn gluten meal upon the incidence of the white feather syndrome. The diets and a summary of the observations are shown in table 1. The feather pigmentation was judged on an arbitrary scale when 0 indicated normal color, 1 indicated slightly abnormal extent of white color on the feathers of a few poults in the group, 2 indicated all poults with slight degrees of pigmentation failure, 3 indicated moderate to severe loss of pigment, and 4 indicated very severe loss of pigment with extremely wide white bands on both wings and also on the tail feathers. Figure 1 shows the normal bronze pattern of the poults on diet 4213 and the abnormal white wing feathers of poults on diet 4215.

The data of Almquist ('45) and of Block and Bolling ('45) on the composition of soybean protein and of corn gluten

protein indicated large differences in the content of glycine and lysine. In the second test, crystalline glycine [1] and crude lactalbumin, respectively, were added to the corn gluten meal diet, no. 4215, in such quantities as to raise the intake

TABLE 1

Observations on corn gluten meal, fiber content, and calcium content as possible causes of pigmentation failure in bronze turkey poults.

INGREDIENTS	DIET					
	4213	4214	4215	4216	4217	4218
	%	%	%	%	%	%
Yellow corn meal	20.4	20.4	20.4	22.9	25.4	17.9
Pulverized oats	10.0	10.0	10.0	5.0	...	10.0
Rolled oats	...	...	...	5.0	10.0	...
Ground wheat	...	...	...	10.0	20.0	...
Standard middlings	10.0	10.0	10.0	5.0	...	10.0
Wheat bran	10.0	10.0	10.0	5.0	...	10.0
Alfalfa meal	7.5	7.5	7.5	3.75	...	7.5
Alfalfa leaf meal	...	...	...	1.25	2.5	...
Meat and bone scrap	12.5	12.5	12.5	12.5	12.5	12.5
Sardine meal	2.5	2.5	2.5	2.5	2.5	2.5
Soybean meal	20.0	10.0	...	20.0	20.0	20.0
Corn gluten meal	...	10.0	20.0	...	...	...
Flaydry [1]	2.0	2.0	2.0	2.0	2.0	2.0
Ladpro 100 D [2]	1.6	1.6	1.6	1.6	1.6	1.6
Ground limestone	2.5	2.5	2.5	2.5	2.5	5.0
Salt with I_2 and Mn	1.0	1.0	1.0	1.0	1.0	1.0
Calculated % Fiber	6.78	6.46	6.14	5.02	3.26	6.73
Calculated % Protein	24.8	25.0	25.2	24.5	24.2	24.6
Calculated % Lysine	1.23	1.02	0.81	1.18	1.13	1.22
No. poults	35	35	35	35	34	34
Av. wt. of poults at 6 weeks (gm)	466	589	605	665	631	464
Achroma score	0	1.5	3	0	0	0

[1] Poultry feed supplement containing whey solubles, used to supply B-complex vitamins.

[2] Poultry feed supplement containing fish solubles and fish liver and glandular meal, standardized to contain 100 A.O.A.C. units of vitamin D per gm.

of these amino acids to the levels which would be supplied if soybean meal had replaced the corn gluten meal. The preventive values of crystalline d-lysine monohydrochloride [2] and

[1] Dow.

[2] Merck.

of calcium pantothenate were also tested in conjunction with the high corn gluten meal diet. Two levels of ground limestone were also used in this series to determine any effect of varying the calcium content of the ration. Table 2 summarizes the diets and observations.

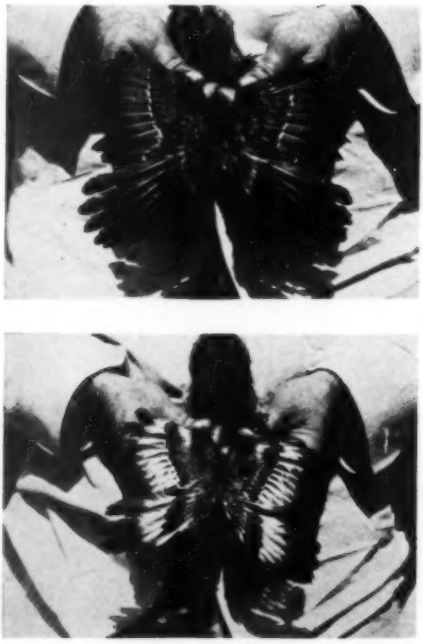

Fig. 1 The upper poult was raised on diet 4213 and shows the normal bronze pattern. The lower poult was raised on diet 4215 and shows the abnormal white wing feathers.

Table 3 summarizes a test in which the effectiveness of crystalline d-lysine (Block) was compared with soybean meal, yeast, and casein as food sources of lysine. Several modifications of the lysine deficient ration were produced by varying the protein sources used. The basal mixture had the following composition: Yellow corn meal 26.3, pulverized oats 10.0, standard middlings 10.0, wheat bran 10.0, dehydrated al-

falfa meal 5.0, Flaydry [3] 2.0, Ladpro 100 D [4] 1.6, ground limestone 2.5, and salt with added iodine and manganese 1.0. To this basal mixture were added the variables of interest indicated in table 3. Diet 4406 contained the least lysine of any diet used in this study.

TABLE 2

Effect of certain supplements added to the corn gluten meal diet which causes feather pigmentation failure.

PEN	SUPPLEMENT TO DIET 4215	CONTENT OF			NO. OF POULTS	AV. WT. AT AGE IN WKS.	ACHROMA SCORE
		Protein	Lysine	Ground Limestone			
		%	%	%		gm	
4269	Glycine, 1.25%	26.1	0.80	2.5	8	377 (5)	4
4270	Lactalbumin, 10.5%	27.3	1.18	2.5	8	396 (5)	0
4271	None	25.2	0.81	2.5	8	355 (5)	3.5
4354	None	25.2	0.81	2.5	6	182 (4)	4
4355	Calcium pantothenate 10 mg/lb	25.2	0.81	2.5	6	240 (4)	4
4356	Lysine, 1.8 gm/lb	25.6	1.20	2.5	6	340 (4)	0
4357	None	24.6	0.79	5.0	6	227 (4)	3
4358	Lysine, 1.8 gm/lb	25.0	1.19	5.0	6	296 (4)	0

The preventive value of fish solubles and of fish meal were investigated, and this test is summarized in table 4. It was considered desirable to study these materials because of their general acceptance and wide use in commercial poultry feeds. Almquist ('45) gives the lysine content of fish solubles as 4.9% of the crude protein, and of fish meal as 5.7% of the crude protein.

DISCUSSION

From the data presented in table 1, it seems obvious that the only cause of the white feather syndrome was the use of corn gluten meal. Among the differences in amino acid composition of soybean meal, which did not produce the syn-

[3] Poultry feed supplement containing whey solubles, used to supply B-complex vitamins.

[4] Poultry feed supplement containing fish solubles and fish liver and glandular meal, standardized to contain 100 A.O.A.C. units of vitamin D per gram.

drome, and of corn gluten meal, which did produce the syndrome, was that of the lysine content. Throughout the course of the tests outlined in this report, the use of protein sources which were good carriers of lysine prevented the pigmentation failure. Whenever proteins of low lysine content were substituted, the white feather syndrome was observed. The use

TABLE 3

Effect of varying the source of protein on the incidence of the white feather syndrome.

INGREDIENTS	DIET								
	4398	4399	4400	4401	4402	4403	4404	4405	4406
	%	%	%	%	%	%	%	%	%
Basal mixture [1]	68.4	68.4	68.4	76.7	68.4	68.4	68.4	68.4	68.0
Meat and bone scrap	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	6.5
Sardine meal	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	..
Cottonseed meal	16.6	..	..	..	..	..	..	..	8.5
Soybean meal	..	16.6	..	..	..	..	..	..	..
Dried brewer's yeast	..	..	16.6	..	..	..	..	..	..
Casein [2]	..	..	..	8.3	..	..	..	..	..
Linseed meal	..	..	..	..	16.6	..	..	..	8.5
Corn gluten meal	..	..	..	..	..	16.6	16.6	16.6	8.5
Lysine, crystalline [3]	..	..	..	..	..	..	1.8[3]	3.6[3]	..
Protein % (calculated)	24.1	24.0	24.9	25.0	23.0	24.3	24.7	25.1	22.4
Lysine % (calculated)	0.92	1.14	1.20	1.24	0.88	0.79	1.18	1.58	0.69
No. of poults	12	12	12	12	12	12	13	13	13
Av. wt. at 5 weeks	317	404	328	357	265	227	394	348	253
Achroma score	1	0	0	0	0	1	1	0	3

[1] See text for composition of basal mixture.

[2] Difference made up with the yellow corn meal.

[3] Lysine additions are expressed as grams per pound. The lysine used was Block's d-lysine. 2 HCl.

of crystalline lysine was as effective in preventing the syndrome as were any natural sources of lysine. It therefore seems obvious that under the conditions of this study, lysine deficiency was the cause of the pigmentation failure. Of course, it must be recognized that not all cases of pigmentation failure are necessarily due to a lysine deficiency. This syndrome is only an indication and as such may have numerous causes.

TABLE 4

Study of fish solubles and fish meal and their effect upon incidence of the depigmentation.

INGREDIENTS	DIET										
	4440	4441	4442	4443	4444	4445	4446	4472	4473	4474	4475
	%	%	%	%	%	%	%	%	%	%	%
Yellow corn meal	..	..	..	56.50	55.50	54.50	52.50	25.90	25.89	25.91	25.90
Ground barley	20.40	18.40	16.40	..	..	..	..	..	..	..	..
Pulverized oats	10.00	10.00	10.00	..	..	..	..	10.00	10.00	10.00	10.00
Standard middlings	10.00	10.00	10.00	..	..	..	..	10.00	10.00	10.00	10.00
Wheat bran	10.00	10.00	10.00	..	..	..	..	10.00	10.00	10.00	10.00
Alfalfa meal	7.50	7.50	7.50	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Meat and bone scrap	12.50	12.50	12.50	..	..	..	..	10.00	10.00	10.00	10.00
Sardine meal	2.50	2.50	2.50	..	..	..	..	2.50	5.00	7.50	10.00
Condensed fish solubles	..	2.00	4.00	..	..	2.00	4.00	..	..	..	..
Glycine	..	..	..	..	1.00	..	..	..	..	..	..
Soybean meal	..	..	..	34.50	34.50	34.50	34.50	..	..	..	..
Corn gluten meal	20.00	20.00	20.00	..	..	..	..	6.50	5.67	4.83	4.00
Cottonseed meal	..	..	..	..	..	..	..	6.50	5.67	4.83	4.00
Linseed meal	..	..	..	..	..	..	..	6.50	5.67	4.83	4.00
Flaydry	2.00	2.00	2.00	..	..	..	..	2.00	2.00	2.00	2.00
Ladpro 100 D	1.60	1.60	1.60	..	..	..	..	1.60	1.60	1.60	1.60
400 D fish oil	..	..	..	0.50	0.50	0.50	0.50	..	..	..	..
Ground limestone	2.50	2.50	2.50	1.00	1.00	1.00	1.00	2.50	2.50	2.50	2.50
Steamed bone meal	..	..	..	2.00	2.00	2.00	2.00	..	..	..	..
Salt with I_2 and Mn	1.00	1.00	1.00	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00
Additional vitamins	..	..	..	(See footnote [1]				..	..	..	..
Protein % (calculated)	25.7	26.3	26.9	20.5	21.4	21.1	21.7	23.2	23.7	24.2	24.7
Lysine % (calculated)	0.81	0.85	0.89	1.04	1.04	1.08	1.12	0.82	0.89	0.95	1.02
No. of poults	10	10	10	10	10	9	9	7	7	7	7
Average weight at 5 weeks	355	384	387	298	295	420	393	220	340	336	350
Achroma score	4	3.5	3.5	2	2	1	0	3	3	2	1

[1] To diets 4443 to 4446, inclusive, the following crystalline vitamins were added per pound: 1.2 mg riboflavin, 68.0 mg nicotinic acid, and 3.4 gm choline chloride.

A reasonable rate of growth is required to bring out the abnormality. If growth is below a critical limit, the lysine requirements are apparently reduced to the point where sufficient lysine is available for pigmentation.

Lysine is also seen to be a growth factor. This confirms the conclusion that the syndrome described is actually a deficiency. In all but a few cases the addition of either a natural source of lysine or of crystalline lysine did stimulate growth as well as prevent the pigmentation failure.

There are a few discrepancies in the growth rate which are not fully explained. The growth on diet 4213 was relatively poor (table 1). However, the average weight shown is influenced considerably by an unusual number of very small poults. If these were eliminated, the average weight of the birds on diet 4213 would be fully up to the highest weights obtained with other diets in this trial.

Calculations of the lysine content of the various rations are based upon data of Almquist ('45) and Block and Bolling ('45). With a few exceptions, chiefly those summarized in table 3, the incidence of the white feather syndrome varied inversely with the lysine content of the ration. Diets which contained 1.1% to 1.2% of lysine seemed to meet the poults' requirements for growth and pigmentation. These figures are above the recommended nutrient allowances for chickens (Cravens et al., '44). It seems apparent that the turkey poult requires considerably more lysine than does the chick. Similar differences have been noted in vitamin requirements.

When poults were placed on a lysine deficient diet, the white feather syndrome became apparent at about 2 weeks of age. The abnormal condition reached a maximum at about 5 weeks of age, and then gradually receded. Even when the poults were kept on diet 4215, the normal bronze pigmentation had nearly completely replaced the white by the time the poults were 10 weeks of age. This is somewhat more rapid disappearance of the syndrome than was noted by Funk and Kempster ('40).

As would be predicted from the lysine content, fish solubles and fish meal showed little protective value. Unless higher than usual levels of these ingredients are used, they are not especially effective in preventing the white feather syndrome. They do, however, show a growth stimulating value which may be due to other factors. Variations in the fiber or calcium content of the ration, and additions of glycine and calcium pantothenate, had no apparent influence on the incidence of the white feather syndrome.

Some of the poults on these diets also developed a curled feather condition similar to the symptoms of vitamin B_{10} deficiency described by Briggs, Luckey, Elvehjem and Hart ('44). This condition did not parallel the pigmentation failure, and may have indicated another deficiency in the rations used.

SUMMARY

A feather pigmentation failure was observed in bronze poults raised in confinement on diets containing a high percentage of certain vegetable protein concentrates. The syndrome was prevented by adding crystalline lysine to the diet, or by substituting protein concentrates high in lysine. The data indicates that the diet must contain approximately 1.1 – 1.2% of lysine for normal feather pigmentation and to permit optimum growth of poults.

LITERATURE CITED

ALMQUIST, H. J. 1945 Proteins and Amino Acids in Animal Nutrition. Booklet published by H. J. Almquist, Dir. of Research, F. E. Booth Co., Inc., San Francisco, Cal.

BLOCK, R. J., AND D. BOLLING 1945 The Amino Acid Composition of Proteins and Foods. C. C. Thomas, Springfield, Ill.

BRIGGS, G. M., JR., T. D. LUCKEY, C. A. ELVEHJEM AND E. B. HART 1944 Further studies on vitamins B_{10} and B_{11} and their relation to "folic acid" activity. J. Biol. Chem., vol. 153, p. 423.

CARRICK, C. W. 1945 Personal communication.

CRAVENS, W. W., H. J. ALMQUIST, L. C. NORRIS, R. M. BETHKE AND H. W. TITUS 1944 Recommended nutrient allowances for poultry. A report by the Sub-committee on Poultry Nutrition, National Research Council.

EWING, W. R. 1943 Handbook on Poultry Nutrition. Rev. ed. W. R. Ewing, S. Pasadena, Cal.

FUNK, E. M., AND H. L. KEMPSTER 1940 The use of vegetable protein concentrates for raising turkeys. Univ. of Mo., Agr. Exp. Sta. Bull. 414

GROODY, T. C., AND M. E. GROODY 1942 Feather depigmentation and pantothenic acid deficiency in chicks. Science, vol. 95 (2478), p. 655.

MCCONACHIE, J. D., W. R. GRAHAM, JR. AND H. D. BRANION 1935 A study of the protein requirements of growing chicks. Sci. Agr., vol. 15, p. 754.

POLEY, W. E. 1938 The utilization of wheat and wheat by-products in feeding young chickens. IV. Ground wheat as a source of vitamin G growth factor. Unknown factors which affect plumage color and structure. Poultry Sci., vol. 17, p. 390.

ROBERTS, R. E. 1945 Personal communication.

SELF SELECTION OF DIET

I. SELECTION OF PURIFIED COMPONENTS [1]

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TWO FIGURES

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On the assumption that preferences for foods are indicative of nutritional requirement and therefore physiologic need, several attempts have been made to determine the effect of varying physiologic requirement on the voluntary intake of purified or partially purified food components by the rat. Under certain conditions, successful choice has been reported (Richter et al., '38a, '38b, '38c, '39, '41a, '41b; Clark and Clausen, '43; Cahill et al., '43). Other investigators have found a limited ability of rats to select proper foods (Kon, '31; Warkentin et al., '43).

The present experiments were designed as an attempt to clarify the role of nutrition in the self selection behavior of rats.

EXPERIMENTAL METHODS

Albino rats of a mixed strain were weaned at 21 to 25 days of age and put in individual cages with four identical cups in a rack, all filled with the standard diet.[2] Vitamins were

[1] Contribution No. 581 of the Department of Chemistry, University of Pittsburgh. Aided by a research grant of the Buhl Foundation.

[2] The standard diet consisted of 24% purified casein (Labco "vitamin-free"), 4% salts (Jones and Foster, '42), 10% hydrogenated fat (Primex), and 62% sucrose.

supplied daily in the form of pills.[3] This diet and supplement have given satisfactory growth in all experiments in this laboratory in which they were used.

The amount of food eaten from each cup was recorded daily, and the cups were then interchanged in a predetermined random manner. After a 3-week control period, the standard diet was replaced by sucrose, Primex, casein, and salts, each in an individual cup. The amount of each component was recorded daily for 3 weeks, and the cups interchanged in the same random manner as before.

RESULTS

Three types of eating behavior were apparent in the 3-week control period. These were: (1) Position eating — The animals preferred to eat from the two cups on the end, as opposed to the two in the middle. As individuals, they usually ate more from either the right or left end cup, and more animals ate from the right than from the left end cup. (2) Repetitious eating — The animals had a tendency to eat from the cup they had eaten from before, in spite of its position or change of position. (3) Random eating — Certain animals ate at random from the cups. This tendency could very properly be considered a lack of the first two types of behavior.

As would be expected from general knowledge of animal behavior, the proportion of animals which showed one type of eating behavior to the exclusion of the others was small. Most animals therefore had tendencies toward all three. A summary of the eating habits during the control period of eighty-seven animals used in this study is shown in table 1. The division into two groups, A and B, is based on growth performance during the experimental period. Group A lost weight and group B gained weight during the latter period.

[3] Each pill contained approximately: 60 μg thiamine hydrochloride; 120 μg riboflavin; 90 μg pyridoxine hydrochloride; 150 μg calcium pantothenate; 10 mg choline chloride; 1 mg α-tocopherol; 55 IU Vitamin A and 11 IU Vitamin D, both as 0.001 ml Natola in a dextrin-powdered sugar base. One pill was offered each rat daily throughout the experiment and, after the first 2 or 3 days, it was consumed avidly.

During the 3-week experimental period all possible types of eating behavior were observed. A few animals ate only sucrose or only fat until they died; some animals alternated from one to the other; some animals ate no protein; others for a fairly long period ate protein exclusively. The only generalization that could be drawn was that no animal disliked all of the choices, and accordingly no indications of caloric deficiency were observed. The only differences of choice in the two sexes were attributable to differences in growth rate and to total amount of food eaten.

TABLE 1

Eating behavior of rats during 3-week control period.[1]

GROUP	NO. OF ANIMALS	GROWTH	FOOD EATEN	% TOTAL FOOD EATEN FROM POSITION [2]			
				(a)	(b)	(c)	(d)
		gm	gm				
A ♂	20	49.5 ± 3.3	108.4 ± 5.4	22.4 ± 1.2	20.4 ± 2.1	24.2 ± 2.1	33.0 ± 2.9
♀	14	45.0 ± 3.6	100.0 ± 5.6	23.5 ± 2.6	19.8 ± 1.9	19.3 ± 1.8	37.6 ± 4.0
All	34	47.0 ± 2.5	105.0 ± 3.9	22.8 ± 1.3	20.2 ± 1.4	22.2 ± 1.5	34.9 ± 2.4
B ♂	28	66.4 ± 2.6	133.8 ± 3.8	27.8 ± 2.3	20.5 ± 1.4	21.5 ± 1.0	28.9 ± 1.6
♀	25	59.7 ± 2.4	126.9 ± 3.4	27.2 ± 2.6	21.3 ± 1.5	21.5 ± 1.1	30.2 ± 1.8
All	53	63.2 ± 1.8	130.5 ± 2.6	27.5 ± 1.7	20.8 ± 1.0	21.5 ± 0.9	29.5 ± 1.8
Total	87	56.9 ± 1.7	120.5 ± 2.6	25.7 ± 1.2	20.6 ± 0.8	21.8 ± 0.8	31.6 ± 1.2

[1] All data in terms of mean and standard error of the mean.
[2] Position (a) was to the animals left while eating.

Because of the marked variability in eating behavior and growth response, the data obtained were difficult to present quantitatively. The frequency distributions of fat, protein, and carbohydrate in terms of per cent by weight of food eaten or per cent of total calories did not approach the normal frequency curve, and it was believed that calculated averages and standard errors would be almost meaningless in these cases. However, a histogram of weight of fat eaten approached the normal distribution; weight of protein and salt gave bimodal distributions, and weight of carbohydrate gave a skewed unimodal distribution (fig. 1). The situation was

further clarified by the division of the animals into two groups on the basis of growth. (Group A lost weight during the experimental period; group B gained weight). Each group showed a frequency distribution sufficiently near the normal to allow the calculation of average values and their standard errors for weights of fat, protein, and salt eaten as well as for weight change and total calories eaten (table 2). Both groups

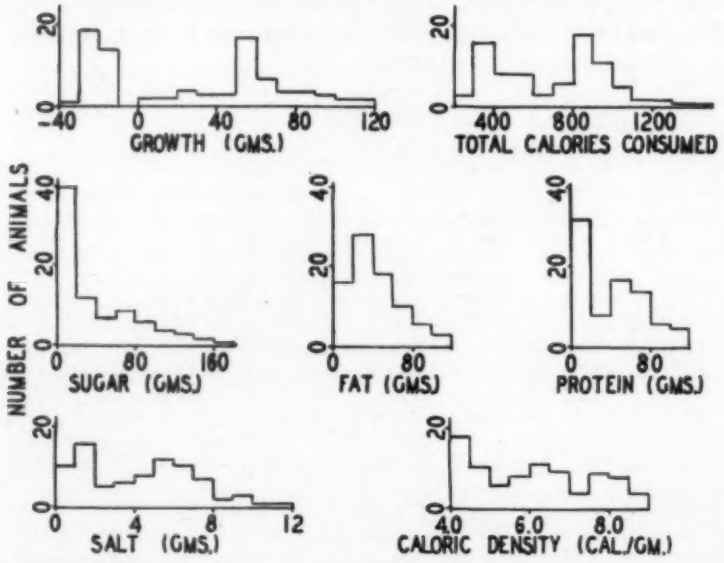

Fig. 1 Distribution of growth and eating habits of rats during a 3-week period of self selection.

showed the same skewed distribution for weight of carbohydrate eaten as is shown in figure 1, and the average figure for weight of carbohydrate presented in table 2 is of doubtful significance. A calculated standard error of weight of carbohydrate would obviously be meaningless under the circumstances.

It will be noted from table 1 that those animals which grew on the selection experiment also grew significantly more during the control period.

Histograms of calculated caloric density (total cal./total weight of food) of diets selected by both group A and group B showed bimodal frequency distributions (fig. 2). A somewhat arbitrary division of the groups on the basis of caloric density resulted in groups which ate (aside from protein and salts) primarily fat or primarily carbohydrate for the balance

TABLE 2

Average selections of animals during 3-week experimental period.

GROUP	NO. OF ANIMALS	CHANGE IN BODY WT.	TOTAL CALORIES EATEN	PROTEIN EATEN	FAT EATEN	SALT EATEN	CARBOHYDRATE EATEN [2]
		gm		*gm*	*gm*	*gm*	*gm*
A ♂	20 [1]	−21.8 ± 1.3	421 ± 28	1.4 ± 0.5	38.3 ± 3.9	0.7 ± 0.1	18.1
♀	14 [1]	−19.8 ± 0.9	382 ± 26	1.3 ± 0.4	30.2 ± 4.5	1.3 ± 0.3	29.8
All	34 [1]	−21.0 ± 0.8	403 ± 19	1.3 ± 0.4	35.0 ± 3.0	0.9 ± 0.2	22.9
B ♂	28	66.6 ± 5.8	920 ± 45	58.5 ± 5.2	50.4 ± 6.9	5.4 ± 0.4	58.1
♀	25	50.4 ± 3.3	829 ± 29	62.0 ± 5.2	40.1 ± 4.2	5.4 ± 0.4	55.0
All	53	58.9 ± 3.6	877 ± 28	60.1 ± 3.7	45.5 ± 4.2	5.4 ± 0.3	56.7

[1] Three males and two females died in group A, presumably due to lack of protein in the diet.

[2] For discussion of significance of this figure, see text.

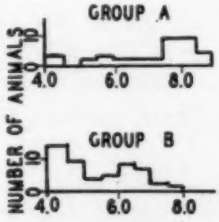

Fig. 2 Distribution of caloric densities of self selected diets.

of their diet. The subdivided groups (table 3) did not differ greatly in other respects. The differences that appear in weight change and total calories of groups B_1 and B_2 in table 3 are in part due to the uneven distribution of the sexes in the two groups.

Rats from fourteen complete litters of varying size were used in this experiment. Table 4 shows the sex distribution and numbers of animals in groups A and B of each litter.

The probability that an arrangement, as improbable as the group distribution, could result from the litter being a random sample of all animals used was calculated.[4] In table 4 it is seen that seven of fourteen litters have probabilities

TABLE 3

Relation of caloric density of self-selected diets to growth, calories and food choice.

		GROUP			
		A_1	A_2	B_1	B_2
Number of animals:	♂	16	5	16	12
	♀	7	6	10	15
Caloric density		8.0 ± 0.1	5.4 ± 0.3	6.3 ± 0.1	4.5 ± 0.1
Change in body weight (in gm)		−21.8 ± 1.0	−19.3 ± 1.3	65.5 ± 5.1	52.7 ± 3.3
Total calories eaten		404 ± 25	400 ± 29	917 ± 38	839 ± 42
Protein eaten (in gm)		1.6 ± 0.5	0.9 ± 0.3	57.0 ± 3.7	63.1 ± 6.3
Fat eaten (in gm)		41.8 ± 2.5	19.7 ± 5.1	70.9 ± 3.8	21.1 ± 2.5
Carbohydrate eaten (in gm)		7.4 ± 1.2	57.3 ± 5.7	12.7 ± 1.4	99.0 ± 7.2
Salt eaten (in gm)		0.8 ± 0.2	1.1 ± 0.4	5.5 ± 0.3	5.3 ± 0.5

less than 0.22. Since the probability of the latter occurring due to random sampling is 1 in 50 (0.020), litter mates must tend to have the same appetites. It is interesting, however, that no litter of four or more animals failed to have members in both groups.

[4] The probability of any one animal being in group A is $34/87 = .39$. The probability P_A that X animals in a random sample of size n will be in group A is:

$$P_A = \frac{n(n-1) \ldots \ldots (n-X+1)}{1 \cdot 2 \cdot 3 \ldots \ldots (n-X)} (.39)^X (.61)^{n-X}$$

The probability that an arrangement as improbable as X of n animals in group A could result from random sampling is P_A plus the sum of the probabilities of all less probable arrangements. The probability P_B of seven of fourteen litters having a probability of less than 0.22 obviously:

$$P_B = \frac{14 \cdot 13 \cdot 12 \cdot 11 \cdot 10 \cdot 9 \cdot 8}{1 \cdot 2 \cdot 3 \cdot 4 \cdot 5 \cdot 6 \cdot 7} (.22)^7 (.78)^7 = 0.015$$

The sum of the probabilities of less probable arrangements of fourteen litters is 0.005, and therefore the probability that an arrangement of fourteen litters could be as improbable as that shown in table 4 because of random sampling is 0.020.

TABLE 4

Litter distribution of animals on self selection.

LITTER	NUMBER OF ANIMALS				PROBABILITY [1]	LITTER	NUMBER OF ANIMALS				PROBABILITY [1]
	(1)		(2)		(3)		(1)		(2)		(3)
	♂	♀	Group A	Group B			♂	♀	Group A	Group B	
D	2	2	2	2	0.646	K	6	4	3	7	0.750
C	3	3	5	1	0.037	L	3	3	1	5	0.415
E	4	2	1	5	0.415	M	3	3	4	2	0.217
F	1	0	1	0	0.390	N	6	2	1	7	0.165
G	6	4	8	2	0.017	O	4	5	1	8	0.177
H	0	1	0	1	1.000	P	3	2	4	1	0.079
I	3	3	2	4	1.000						
J	4	5	1	8	0.177	Total	48	39	34	53	

[1] Probability that a group distribution as improbable as that in column (2) could arise from random sampling.

DISCUSSION

The pronounced variability and atypical frequency distributions of the choices of the rats in this experiment cannot be too strongly emphasized. These characteristics prevent the calculation of averages and standard errors of appetites of small numbers of animals, and limit the usefulness of the self-selection method to large groups.

Under present conditions, the following conclusions can be reached:

1. Rats either do or do not like casein; if they like it, they eat an average of 3 gm per day and grow well; if they do not, they eat less than 0.1 gm per day, lose weight, and die within a short period.

2. Rats have a uniform appetite for hydrogenated fat which varies only slightly with the growth rate, and therefore with the total calories eaten.

3. A large proportion of rats eat very little sucrose, while only a few eat it in large amount.

4. Rats which grow eat about 0.25 gm of salts per day; rats which do not, eat 0.05 gm per day.

5 Rats which like casein grow significantly better on a standard diet than do rats which dislike casein.

6. Litter mates tend to have similar appetites, indicating the influence of hereditary factors on choice.

Two necessary criteria that an appetite be based on a dietary need are: (a) The need must be accompanied by an appetite. (b) A change in need must be accompanied by a change in appetite. These are not sufficient criteria, however, to prove the point because the animal may learn by experience that a particular choice gives him a greater feeling of satisfaction, while obviously he is unable to recognize a dietary need as such.

It is in the light of these two criteria that the dietary significance of this experiment becomes most apparent. Since all the rats had a need for protein, and only fifty-three out of eighty-seven had an appetite for it, there does not appear to be a relation between need and appetite in this case. It is even possible that the appetite for protein of the animals in group B may be based on a trivial factor such as taste, consistency, or desire for variety. The widely varied appetites for fat and carbohydrate seem to have no basis in dietary need.

The appetite for salts does seem to be related to need. With few exceptions, the animals which exhibited growth selected salts and those which did not ate very little. There was no evidence of growth retardation due to low intake of salts. Similarly, the appetite for calories seemed to be related to need. No animal failed to grow because of lack of calories, and no animal lost weight unusually rapidly because of failure to eat. The type of food eaten to supply calories, aside from protein, had little effect on total calories eaten, as shown in table 3, and the only appetite which affected growth was that for protein.

The most probable conclusions under the conditions of this experiment appear to be: (a) The only nutritional requirements related to eating behavior are need for salts and need for calories. (b) The form in which calories are eaten is independent of need. (c) The need for salts and calories is

determined by the protein intake, which, of itself, is independent of need.

Finally, it is common knowledge that dietary requirements of a group of animals, like all physiologic measurements, can be described as a mean plus or minus a small standard error. The wide variability of the appetites here demonstrated can be considered as an additional indication that they are not based on dietary needs, for the latter show much less variability.

The fact that rats which are able to make a successful choice also grow better on a standard diet is of special interest. If a similar correlation exists in the case of hogs, the explanation of the good growth of these animals on self-selection feeding may be the fact that they have been bred for many generations for growing ability.

The results here presented are appreciably different from those presented in several of the studies mentioned above. Possible explanations of differences in these and earlier results may be as follows: (1) In some reports, the number of animals was too small to draw any significant conclusions. (2) Rats of a different strain were used. (3) Some of the choices presented were not purified foods. For example, a large portion of the protein requirement of rats in certain experiments has been provided by yeast.

SUMMARY

1. The appetites of eighty-seven normal rats, allowed their choice of sucrose, casein, hydrogenated fat, and salts, are described. Thirty-four of these animals failed to grow.

2. The animals could be separated into two groups on the basis of their appetites for casein.

3. The appetites of these animals, aside from that for salts and calories, showed no apparent relation to physiologic or nutritional need.

LITERATURE CITED

CAHILL, W. M., W. F. DUNNING AND A. H. SMITH 1943 A free choice dietary study of tumor bearing rats. Cancer Research, vol. 3, p. 830.

CLARK, W. G., AND D. F. CLAUSEN 1943 Dietary "self-selection" and appetites of untreated and treated adrenalectomized rats. Am. J. Physiol., vol. 139, p. 70.

JONES, J. H., AND C. FOSTER 1942 A salt mixture for use with basal diets either low or high in phosphorus. J. Nutrition, vol. 24, p. 245.

KON, S. K. 1931 The self-selection of food constituents by the rat. Biochem. J., vol. 25, p. 473.

RICHTER, C. P., AND B. BARELARE, JR. 1938a Nutritional requirements for normal growth and reproduction in rats studied by the self-selection method. Am. J. Physiol., vol. 121, p. 185.

———— 1938b Nutritional requirements of pregnant and lactating rats studied by the self-selection method. Endocrinology, vol. 23, p. 15.

———— 1939 Further observations on the carbohydrate, fat and protein appetites of vitamin B deficient rats. Am. J. Physiol., vol. 127, p. 199.

RICHTER, C. P., AND C. D. HAWKES 1941a The dependence of carbohydrate, fat and protein appetite of rats on the various components of the vitamin B complex. Am. J. Physiol., vol. 131, p. 639.

RICHTER, C. P., L. E. HOLT, JR., B. BARELARE, JR. AND C. D. HAWKES 1938c Changes in fat, carbohydrate and protein appetite in vitamin B deficiency. Am. J. Physiol., vol. 124, p. 596.

RICHTER, C. P., AND E. C. H. SCHMIDT, JR. 1941b Increased fat and decreased carbohydrate appetite of pancreatectomized rats. Endocrinology, vol. 28, p. 179.

WARKENTIN, J., C. WARKENTIN AND A. C. IVY 1943 The effect of experimental thyroid abnormalities on appetite. Am. J. Physiol., vol. 139, p. 139.

VARIABILITY IN THE CALCIUM METABOLISM AND CALCIUM REQUIREMENTS OF ADULT HUMAN SUBJECTS[1]

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Variability is the most prominent characteristic of living beings. Variability among the individuals of the same zoological (or botanical) class or subdivision is spoken of as individuality, and the biological basis of individuality has been considered in great detail recently by Loeb ('45). But in the same individual, variability operates in functional behavior from time to time, even under the same environmental conditions, insofar as these conditions can be controlled in biological experimentation. The universality of variation, both morphological and physiological, among and within individual organisms, complicates the planning of experimental investigations, the interpretation of the results secured, and their application to the practical problems of life.

The prevalence of variation in every type of biological study necessitates in most cases the application of statistical analysis in order to distinguish the changes in form or function that may result from changes in conditions deliberately imposed in the plan of the experiment, from the changes in form or function that occur regardless of these changed conditions

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due to the uncontrolled factors in the experiment and to the individuality of the subject organisms.

In physiological and biochemical studies on man and laboratory animals, the prevalence of functional variability under conditions as well controlled as is feasible at present has been pointed out from time to time. The publications of Rietz and Mitchell ('10), Grindley and Mitchell ('17), Bauer, Allbright and Aub ('29), McCance and Widdowson ('42, '43), and Macy ('42), among many others, may be cited in this connection. The variation in biological assays of pharmaceutical products and the service of statistical methods of analysis in dealing with the situation have been ably discussed by Bliss ('41).

However, in nutritional research, the fact that variation in experimental data must be considered and measured for purposes of description and valid interpretation is not always recognized. The simplest mathematical manipulation, the computation of an average, is often the only statistical method used in reducing data to a form from which conclusions are deduced. The process of adding individual data together and dividing by their number, to obtain a value that generally is not identical with any of the measurements actually made, is carried out apparently in a routine way, with little consideration of the consequences of substituting an average for the data from which it was derived, or of its implications. Food composition tables that are so important in practical nutrition in implementing nutritional principles and generalizations, contain only average analyses. Such an abbreviated presentation, often of a rich volume of data, fails to reveal the reliability of different foods as sources of nutrients. It misleads the user of such tables to class together, as a source of calcium for example, a reliable food such as milk with an utterly unreliable food, such as lettuce, merely because their average contents of calcium for the particular samples analyzed happen to be nearly the same.

Similarly, in the presentation of nutritive requirements, averages only are given in the majority of cases. One common use to which such averages are put is the evaluation of the

nutritive status of individuals and populations whose nutritive intake has been more or less accurately estimated. But with no information as to the variability in requirements, the significance of deviations between the average requirements and the estimated intakes cannot be judged on any rational basis. Whether the deviations are within the range of normal requirements, or whether they indicate definite over- or undernutrition cannot be decided, although unfortunately decisions are often made nevertheless. Pett ('43, '45) has ably remonstrated against such a use of requirement data and has strongly advocated the determination of variation in nutritive requirements, as well as mean requirements. Fertig ('43) and Dann and Darby ('45) have presented similar arguments. Kline and Eheart ('44) have reported extreme variations in the ascorbic acid requirements for saturation, even in a small group of women subjects, while Steggerda and Mitchell ('41) have observed a variability of about 20% (coefficient of variation) in the calcium requirements of a small group of men.

It will be the purpose of this paper to report the results of calcium metabolism studies on a group of nineteen men with particular reference to the variation among and within individuals with reference to the paths of excretion and the utilization of ingested calcium and to the indicated requirements of dietary calcium for the maintenance of calcium equilibrium.

EXPERIMENTAL PROCEDURES

There were nineteen subjects in the experiments reported in this and the following paper, which extended over a period of 5 years. Their average age, weight, and height are recorded in table 1, together with estimated surface areas and basal metabolism.

The general plan of the experiment in testing the utilization of the calcium in various milk products and calcium salts was to place each subject upon a basal diet containing on the average 229 mg of calcium daily. This diet contained sufficient food energy to maintain body weight and, with daily supplements of vitamins A and D, it was presumably adequate in all

respects but calcium. After 4 or 5 days on a constant intake of food, collections of feces and urine were made in successive 4-day periods until it was apparent that the subject was adjusted to this low level of calcium nutrition. Carmine was used as a feces marker.

In succeeding metabolism periods, supplements of milk products and calcium salts were added to the basal diet in

TABLE 1

Description of experimental subjects.

SUBJECT	AGE	BODY WEIGHT	HEIGHT	BODY SURFACE [1] AREA	BASAL METABOLISM [2]
	years	*kg*	*cm*	m^2	*Cal*
Steg	39	81	180	2.01	1817
Ne	28	74	184	1.96	1815
Cl	27	70	176	1.86	1727
Fo	25	67	171	1.78	1675
Step	25	83	171	1.95	1895
Haa	25	72	204	2.09	1909
Ve	22	73	186	1.97	1852
Sc	22	87	186	2.12	2045
McB	24	64	181	1.82	1690
Be	28	67	169	1.77	1644
Te	26	67	179	1.84	1708
Sn	23	72	181	1.92	1807
McC	20	63	179	1.80	1694
Sw	20	65	176	1.80	1706
On	20	67	173	1.80	1719
To	29	62	167	1.70	1559
Hal	19	66	166	1.73	1677
Le	17	63	166	1.70	1650
Ro	24	70	186	1.93	1797

[1] Computed by the formula of DuBois and DuBois ('16).

[2] Estimated by the Harris-Benedict (Carpenter, '39) formula.

amounts to provide enough calcium for approximate equilibrium. The milk products tested included pasteurized whole milk, dried defatted milk, dried whole milk, homogenized milk, a commercial soft curd milk prepared by the process of base exchange (Lyman et al., '33), milk heated to 160°F. for 30 minutes and milk with varied supplements of sodium alginate (2%), potassium or sodium citrate, citric acid (0.27%),

and orange juice. The calcium salts used were the citrate and the gluconate. During those periods involving milk supplements, small adjustments were made in the basal diets to equalize the energy intakes in basal and test periods. All experimental periods consisted of a succession of 4-day collection periods continued until it was evident that an adjustment to the experimental diet had occurred over at least 12 days, and generally 20 days or more. Results of a 4-day period at the beginning of a test that indicated incomplete adjustment were not included in the average.

Further details of the experimental procedure are contained in a previous article (Steggerda and Mitchell, '39).

EXPERIMENTAL RESULTS

The average results for each subject on the low-calcium basal diet are summarized in table 2. The averages include all

TABLE 2

Calcium metabolism at low intake levels.
Averages expressed in milligrams per day.

SUBJECT	PERIODS	TOTAL DAYS	CALCIUM INTAKE	CALCIUM IN FECES	CALCIUM IN URINE	CALCIUM BALANCE
Ne	4	84	229	268	60	— 99
Fo	4	60	236	284	79	—127
Steg	11	192	204	216	78	— 90
Cl	2	32	198	204	189	—195
Step	3	56	217	274	77	—134
Has	2	40	208	206	86	— 84
Ve	1	24	221	182	144	—105
Se	2	44	248	273	112	—137
Ro	1	16	201	232	177	—208
Te	1	20	216	307	76	—167
Be	1	20	213	274	65	—126
Sn	5	104	245	247	134	—136
McC	1	20	261	280	237	—256
Sw	1	20	261	150	171	— 60
On	1	20	261	233	100	— 72
To	5	108	195	89	210	—104
Hal	1	20	225	185	175	—135
Le	1	20	225	196	28	+ 1
McB	1	20	281	256	172	—147

of the low-calcium periods for each man, ranging from 1 to 11, and from 16 to 192 days. For twelve of the nineteen subjects, the fecal calcium exceeded the intake, and for seventeen of the subjects, the fecal calcium exceeded the urinary calcium. For eight of the subjects, the urinary calcium averaged less than 100 mg daily, but for six of them, it approximated or exceeded the fecal calcium. These relations testify to the prominence of individuality within this group of men with reference to path of excretion of ingested calcium. The calcium balances are not inversely correlated with the fecal calcium, and for this and other reasons to be developed in the following paper, the authors do not believe that fecal calcium is entirely, or even largely, composed of ingested calcium that has escaped absorption from the gastro-intestinal tract.

The standard deviation of the 212 4-day calcium balances about the means for their respective subjects was 46.3 mg per day. This value measures the functional variability of the subjects while subsisting on essentially the same low-calcium diet. It does not include the variation existing among different subjects, a variation that seems impossible to assess surely from these data, because of the lack of any rational method of correcting calcium balances either for differences in calcium intake or for differences in size of subject. If these differences may be neglected as possessing only minimal effects within the ranges of calcium intake and body size prevailing, the standard deviation of the nineteen average calcium balances is 57.2 mg per day, the average balance being — 125 mg per day.

These standard deviations express in a quantitative fashion the great variation existing within the same individual, but particularly among different individuals, in the disposal of a low level of dietary calcium, in the utilization of dietary calcium in the sparing of body calcium, and possibly in the rate of the endogenous excretion of calcium. It will be noted in particular that subject Le for a period of 20 days was in calcium equilibrium on an intake of 225 mg per day. In contrast, subject McC, somewhat larger than Le but with a calcium intake also 36 mg larger daily, exhibited a negative

balance of 256 mg daily, again over a 20-day period. These extremes in balance deviate from the mean balance for all subjects by only slightly more than twice the standard deviation of 57 mg, and hence they must be considered as resulting merely from a random combination of uncontrolled factors operating throughout the entire group of subjects.

The balance data secured at the higher levels of calcium intake, averaging 545 mg daily, have been pooled together for each subject, regardless of the nature of the supplemental calcium. We have felt justified in doing this, because an intensive study of the data has not revealed any statistically significant differences in utilization of the different supplements of calcium, that is, differences in their sparing effects on body calcium, possibly due in part to the great variation in metabolic performance of the human organism. In support of this statement, the following average percentage utilizations [2] for the various supplements may be cited:

Milk alone, 34% in 24 tests; milk + sodium citrate or citric acid, 34% in 13 tests; milk heated to 160°F. for 30 minutes, 35% in 4 tests; milk + sodium alginate, 29% in 3 tests; dried milk, whole or defatted, 19% in 6 tests; milk + orange juice, 34% in 4 tests; soft curd milk (base-exchange), 29% in 9 tests; homogenized milk, 24% in 4 tests; calcium citrate, 30% in 5 tests; and calcium gluconate, 25% in 4 tests.

It may be thought that the calcium in the dried milk preparations was definitely less well utilized than that of the other supplements. However, the average of 19 is derived from six determinations, two of which were abnormally low, one, in fact, being negative. If these two values are disre-

[2] The calculation of a percentage utilization of calcium may be illustrated from the following data for subject Step:

Low-calcium period	217 mg Ca intake	—134 mg Ca balance
Same plus milk and Na citrate	458 mg Ca intake	— 57 mg Ca balance
Differences	241 mg Ca	77 mg Ca

$(77 \div 241) \times 100 = 32\%$ utilization.

garded, the average becomes 28. The results on the soft curd (base-exchange) milk confirm those of Hess et al. ('40) on infants.

Considering all supplemental sources of calcium as statistically indistinguishable in adult human metabolism, the standard deviation of the 308 4-day calcium balances at the higher level of intake, about the means for their respective subjects, was 59.5 mg per day, a value that measures the functional variability of the subjects while subsisting on a level of calcium approximating that required for equilibrium. A similar standard deviation relating to the percentage utilizations is 9.85%. The latter value refers, however, not to 4-day periods, but to the periods averaging about 20 days in length, employed in the determination of calcium utilization. Evidently, with a standard deviation of this magnitude, it would be difficult to establish the reality of a difference in calcium utilization in the same subject of less than 18.5 percentage units (P=.030), using a test period of 20 days. With a number of subjects, rather than one, this critical difference would be diminished in proportion to the square root of the number used.

The pooled data for each of the nineteen subjects for calcium metabolism at the higher level of intake, together with individual estimates of calcium utilization and of calcium requirement for equilibrium, are summarized in table 3. The average utilization of calcium, computed according to footnote 2, is 31.6. This value would relate to a diet in which about 60% of the calcium is provided by milk, or by calcium salts approximately as well utilized in adult metabolism as the calcium of milk. The standard deviation of individual utilization percentages is 7.36, and the coefficient of variations, 23.3%. The mean value of 31.6% is very nearly the same as that previously reported (Steggerda and Mitchell, '41) for a group of nine men, three of whom were included in the present study. The average calcium utilization reported in the prior study was 29%.

TABLE 3

Calcium utilization and indicated requirements at borderline levels of intake.

SUBJECT	NUMBER OF PERIODS	TOTAL	CALCIUM INTAKE	CALCIUM BALANCE	CALCIUM UTILIZATION	CALCIUM REQUIREMENTS PER DAY			
						Total	Per kg body weight	Per m²	Per basal cal
		days	*mg*	*mg*	%	*mg*	*mg*	*mg*	*mg*
Ne	11	208	649	− 18	19	739	9.99	377	.407
Fo	10	188	634	+ 17	36	588	8.78	330	.351
Steg	14	256	573	+ 5	26	554	6.84	276	.305
Cl	8	160	652	− 48	32	800	11.43	430	.463
Step	1	20	458	− 57	32	636	7.66	326	.336
Has	1	20	430	− 50	16	739	10.26	354	.387
Ve	2	40	589	− 4	27	604	8.27	307	.326
Se	4	84	668	− 19	28	736	8.46	347	.360
Ro	1	20	516	−111	31	876	12.51	454	.487
Te	1	20	498	− 67	35	686	10.24	373	.402
Be	1	20	532	− 36	28	660	9.85	373	.401
Sn	7	152	600	− 44	26	771	10.71	402	.427
McC	1	28	646	−126	34	1018	16.16	566	.601
Sw	3	72	438	+ 17	43	399	6.14	222	.234
On	1	28	465	+ 20	45	421	6.28	234	.245
To	3	60	421	− 26	35	492	7.94	289	.316
Hal	2	40	568	− 28	31	658	9.97	380	.392
Le	2	40	408	+ 66	35	222	3.52	131	.135
McB	2	40	619	− 9	41	641	10.02	352	.379
Means		20	545	− 25	31.6	644	9.21	343	.366
Standard deviations					7.36	181	2.71	93.8	.101
Coefficients of variation, pct.					23.3	28.1	29.4	27.3	27.5

The indicated calcium requirements, computed as in a previous publication (Steggerda and Mitchell, '41)[3] averaged 644 mg per man per day, with a standard deviation of 181 mg. These daily requirements were each expressed per kilogram of body weight, per m^2 of body surface, and per basal calorie in an attempt to eliminate the effect of variable body size. The mean values obtained were 9.21 mg of calcium per kilogram of body weight, 343 mg per m^2, and 0.366 mg per basal calorie. However, it is worthy of note that the coefficients of variation were the same (about 28%) for all practical purposes, whether the values per subject, or per unit of weight, surface, or basal metabolism are considered. In other words, these attempts to eliminate the effect of variable body size were unsuccessful. We do not think, with Leitch ('37), that this means calcium requirements bear no relation to body size. This to us is inconceivable. Rather, it probably means that other factors are so much more potent in causing variation in calcium metabolism as to completely obscure the effect of variable body size.

The calcium requirements summarized in table 3 may be compared with others obtained in a similar fashion and relating also to diets whose calcium contents are derived predominantly from dairy products. Such comparisons are assembled in table 4.

The values for the average total daily requirement of calcium in the four experiments compared exhibit a truly remarkable agreement, being grouped closely about the grand average of 653 mg. Reduced to the per kg and to the per m^2 basis, this total requirement becomes 9.99 mg and 365 mg, respectively. In good agreement also is the average of 9.75 mg of calcium per kilogram of body weight per day computed by Mitchell and Curzon ('39) from selected experiments in the older literature. As in the experiment reported in this

[3] An illustration of the method of computation may be taken from subject Ne. On the basal diet containing 229 mg of calcium daily, the average calcium balance was —99.0 mg. With an average utilization of calcium of 19.4% for this man, the negative balance would be wiped out on feeding 99.0 ÷ .194 = 510 mg additional calcium, or a total of 229 + 510 = 739 mg daily.

TABLE 4

Comparison of average daily calcium requirements in different experiments, determined by the same method with diets containing 53 to 71% of their calcium in milk or equivalent products.

AUTHORITY	NUMBER OF SUBJECTS	TOTAL		PER KG		PER M^2	
		Mean mgs	Coefficient of variation [1]	Mean mgs	Coefficient of variation [1]	Mean mgs	Coefficient of variation [1]
			%		%		%
Steggerda and Mitchell ('41)	9 [2]	657	17.8	9.55	21.6	357	19.1
Breiter et al. ('41)	7 [3]	662	23.1	10.70	16.9	387	16.9
This report	19 [3]	644	28.1	9.21	29.4	343	27.3
Unpublished [4]	8 [5]	664	18.7	11.61	15.5	408	15.0
Total population [6]	*43*	*653*	*22.5*	*9.99*	*23.1*	*365*	*21.8*

[1] The coefficient of variation is the standard deviation expressed as a percentage of the mean.

[2] All men.

[3] Four women, 3 men.

[4] Calcium metabolism studies carried out jointly by the Division of Foods and Nutrition and the Division of Animal Nutrition, University of Illinois.

[5] All women.

[6] Disregarding the variation among the mean results in each experiment.

paper, the variation in individual requirements is about 22%, and is essentially the same for all three methods of expression.

The average daily calcium requirement for equilibrium of adult humans of 10 mg per kilogram seems to be well established by the experiments cited in this paper for individuals of the economic and social class studied, i.e., college students and staff members. It would seem to be a safe value to use in the planning of diets containing an average proportion of calcium from dairy products for groups of individuals. For such cases, there is no good reason for boosting the value of any arbitrary "margin of safety," any more than there is for a requirement of food energy. Apparently, the only good reason for incorporating a "margin of safety" in one case but not in the other, is that, with calcium, as contrasted with energy, an excess intake above the requirement can be tolerated without detriment to the nutriture of the individual and with no great strain on any physiological mechanism. In the dieting of individuals, a daily intake of 10 mg of calcium per kilogram of body weight would be more than enough for some and not enough for others. It is not improbable, however, that the latter group, in the course of time, could adapt themselves to the average intake of calcium. It would seem improvident and even impractical, to feed all adults a level of calcium that would cover the needs of practically all individuals, say all but 1 in 100. This would require an excess above the average of 2.33 times the standard deviation, or a total of 15.13 mg per kilogram of body weight per day, or 1.06 gm per day for a 70-kg man. To cover the needs of all but 1 in 50, the total intake of calcium should be 14.51 mg per kilogram per day, or for 70-kg man, a total intake of 1.01 gm per day.

In judging the adequacy of the calcium nutrition of a group of men, and even more so of an individual, an average requirement of calcium is of little value in itself. The variation to which it is subject, of the order of 22% in terms of the coefficient of variation is sufficient indication of its unreliability. Added to this uncertainty, is the random error to which a

coefficient of variation is subject. A coefficient of 22 obtained from a sample of 43, has a standard deviation of 2.48 (Davenport and Ekas, '36, p. 37). The probability of a considerable power of adaptation in coping with an initially inadequate calcium intake (Mitchell, '44; Kraut and Wecker, '43) must also be reckoned with. These considerations would indicate that a mere determination of the calcium intake of an individual or a community, no matter how carefully it may be accomplished, cannot in itself establish a condition of calcium under-nourishment in the adult. It may, however, afford supporting evidence in conjunction with clincal or laboratory findings indicative of a progressive osteoporosis or some other symptom that can logically be associated with an inadequate level of dietary intake.

SUMMARY AND CONCLUSIONS

The results of calcium metabolism studies on nineteen men, designed to afford information on the utilization of the calcium in diets containing milk products, or equally available calcium salts, to furnish about 60% of the total calcium intake, and the daily requirements of calcium, are reported in this paper. The diet periods averaged 20 days in length and a total of seventy-five such periods are considered in this report. The data appear to support the following conclusions:

1. The calcium of the experimental diets was on the average utilized to the extent of about 32% in the prevention of endogenous losses. The coefficient of variation of this average value is 23.3%.

2. The heating of milk to 160°F. for 30 minutes, the homogenization of milk, the addition of sodium alginate, citric acid or citrates in moderate amounts, the preparation of a soft curd milk by base-exchange, produced no demonstrated change in the utilization of its content of calcium.

3. The average calcium requirement of the adult human indicated by these experiments is 644 mg daily, or 9.21 mg per kilogram of body weight per day, or 343 mg per m^2 per day,

the coefficients of variation being, respectively, 28.1, 29.4 and 27.3.

4. The effect of body size on calcium requirements in adults, for a moderate range in size, is insignificant in comparison with other causes of variation.

5. In conjunction with other experiments of similar nature, involving a total of forty-three subjects, a good average value for the calcium requirement of adult men and women of a nutritional status representative of college students and staff members, subsisting on diets containing dairy products to furnish from one-half to two-thirds of the calcium content, is 10 mg per kilogram of body weight per day. This average is associated with a coefficient of variation of 22 ± 1.7.

6. Such an average is of value in the planning of diets, but of only subsidiary value in forming decisions as to the prevalence of calcium undernutrition in a community. Only in association with clinical or laboratory findings of symptoms indicative of a progressive draining of minerals from the bony structures, or the soft tissues of the body can a marked deficit of intake below an average requirement of calcium implicate this essential nutrient. The great variability exhibited by the human organism in its disposal of dietary calcium, involving, probably, a marked ability to adapt itself to wide ranges in calcium supply, is responsible for this situation.

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LITERATURE CITED

BAUER, W., F. ALLBRIGHT AND J. C. AUB 1929 Studies of calcium and phosphorus metabolism. II. The calcium excretion of normal individuals on a low calcium diet, also data on a case of pregnancy. J. Clin. Invest., vol. 7, p. 75.

BLISS, C. I. 1941 Biometry in the service of biological assay. Ind. Eng. Chem. Anal. Ed., vol. 13, p. 84.

BREITER, H., R. MILLS, J. DWIGHT, B. MCKEY, W. ARMSTRONG AND J. OUTHOUSE 1941 The utilization of the calcium of milk by adults. J. Nutrition, vol. 21, p. 351.

CARPENTER, T. M. 1939 Tables, factors and formulas for computing respiratory exchange and biological transformations of energy. Carnegie Inst. of Washington Publ. no. 303a, 142 pp.

DANN, W. J., AND W. J. DARBY 1945 The appraisal of nutritional status (nutriture) in humans with special reference to vitamin deficiency diseases. Physiol. Rev., vol. 25, p. 326.

DAVENPORT, C. B., AND M. P. EKAS 1936 Statistical methods in biology, medicine and psychology. John Wiley and Sons, New York, 4th ed., 216 pp.

DUBOIS, D., AND E. F. DUBOIS 1916 Clinical calorimetry. X. A formula to estimate the approximate surface area if height and weight be known. Arch. Intern. Med., vol. 17, p. 9.

FERTIG, J. W. 1943 Proceedings of the Research Conference on the Relation of Nutrition to Public Health. The Nutrition Foundation, New York, p. 50.

GRINDLEY, H. S., AND H. H. MITCHELL 1917 Studies in Nutrition. An investigation of the influence of saltpeter on the nutrition and health of man with reference to its occurrence in cured meats. Volume I. Discussion and interpretation of the biochemical data. Chapter III. University of Illinois, 544 pp.

HESS, J. H., H. G. PONCHER, H. W. WADE AND J. C. RICEWASSER 1940 Cow's milk treated by base exchange for infant feeding. Metabolism of calcium, phosphorus and nitrogen. Am. J. Dis. Child., vol. 60, p. 535.

KLINE, A. B., AND M. S. EHEART 1944 Variation in the ascorbic acid requirements for saturation of nine normal young women. J. Nutrition, vol. 28, p. 413.

KRAUT, H., AND H. WECKER 1943 Kalk Bilanz und Kalk Bedarf. Biochem. Z., vol. 315, p. 329.

LEITCH, I. 1937 The determination of the calcium requirements of man. Nutr. Abst. and Rev., vol. 6, p. 553.

LOEB, L. 1945 The biological basis of individuality. Charles C. Thomas, Springfield. Ill., 711 pp.

LYMAN, J. F., E. H. BROWNE AND H. E. OTTING 1933 Readjustment of salts in milk by base exchange treatment. Ind. Eng. Chem., vol. 25, p. 1297.

MCCANCE, R. A., AND E. M. WIDDOWSON 1942 Mineral metabolism of healthy adults on white and brown bread dietaries. J. Physiol., vol. 101, p. 44.

———— 1943 Seasonal and annual changes in the calcium metabolism of man. J. Physiol., vol. 102, p. 42.

MACY, I. G. 1942 Nutrition and chemical growth in childhood. Volume I. Evaluation: Chapter VIII. Metabolic balances. Charles C. Thomas, Springfield, Ill., 432 pp.

MITCHELL, H. H. 1944 Adaptation to undernutrition. J. Am. Diet Assn., vol. 20, p. 511.

MITCHELL, H. H., AND E. G. CURZON 1939 The dietary requirement of calcium and its significance. Actualities Scientifiques et Industrielles, no. 771, Nutrition XVIII, Hermann and Cie, Paris, 101 pp.

PETT, L. B. 1943 Proceedings of the Research Conference on the Relation of Nutrition to Public Health. The Nutrition Foundation, New York, p. 22.

———— 1945 Errors in applying nutrient allowances to dietary surveys or food policies. Canadian J. Pub. Health, vol. 36, p. 69.

RIETZ, H. L., AND H. H. MITCHELL 1910 On the metabolism experiment as a statistical problem. J. Biol. Chem., vol. 8, p. 297.

STEGGERDA, F. R., AND H. H. MITCHELL 1939 The calcium requirement of adult man and the utilization of the calcium in milk and in calcium gluconate. J. Nutrition, vol. 17, p. 253.

———— 1941 Further experiments on the calcium requirement of adult man and the utilization of the calcium in milk. J. Nutrition, vol. 21, p. 577.

THE EFFECT OF THE CITRATE ION ON THE CALCIUM METABOLISM OF ADULT HUMAN SUBJECTS[1]

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The literature on factors affecting the utilization of dietary calcium in the animal body is peculiarly conflicting, whether one considers the effect of intestinal reaction, fat, fiber, phytic acid or lactose. Citric acid, also, is no exception to this statement. Among many other organic acids, it is very completely oxidized in the body (Lanford, '42 b; Metcalf and Hathaway, '45). It is a normal constituent of bone (Dickens, '41; Class and Smith, '43). The blood citrate may constitute a physiological mechanism for the removal of calcium (Lansing and Scott, '42) and lead (Kety, '42) from the tissues and their excretion in the urine (Shorr et al., '42). Cattle and rabbits given sodium citrate intravenously, or by stomach tube, have been observed to increase their output of calcium in the urine (Marek, Wellmann and Urbányi, '42).

Dietary citrates (Hamilton and Dewar, '37), or a mixture of citric acid and citrates (Shohl, '37; Shohl and Butler, '39), have been found beneficial in the prevention or treatment of rickets, but Day ('40) claims that, in the rat at least, these beneficial effects do not develop unless there is a high calcium to phosphorus ratio in the diet, and appreciable quantities of phytin or perhaps other phosphorus-containing complexes of

[1] This investigation was aided by funds contributed to the University of Illinois by the Nutrition Foundation, Inc.

low biological availability. Hathaway and Meyer ('39), working with rachitic rats and large doses of citrates equivalent to over 10% of the diet, claimed special potency for potassium citrate over the sodium salt, and failed to confirm a need of a mixture of citrates with citric acid.

In normal animals and children, the effect of citrates on calcium metabolism has not been clarified by available information. Chaney and Blunt ('25) reported that in two girls, 10 and 11 years old, calcium assimilation was decidedly benefited when 600 to 700 ml of orange juice formed a part of the diet, but the general nutritional benefits in increased weight and nitrogen retention following the incorporation of orange juice in the diet suggest that other factors than citric acid were operating. Lanford ('39) has reported similar, though not so marked, effects of orange juice on calcium assimilation in the growing rat, but in later experiments ('42 a), doses of citric acid and potassium citrate equivalent to those contained in the amounts of orange juice fed, were without appreciable effect upon calcium retention. Also, Mallon and Lord ('42) observed no favorable effect of lemon juice on the calcium retention of growing rats, although lemon juice contains six times as much citric acid as orange juice (Hartmann and Hillig, '34). Watson, McGuire, Meyer and Hathaway ('45) failed to observe any demonstrable effect of either orange juice, ascorbic acid or potassium citrate (3.38 gm daily) on the calcium retentions of eight pre-school children in experiments extending over 16 weeks.

The low efficiency with which the human organism, and especially the adult organism, assimilates dietary calcium, even when milk provides two-thirds of the calcium consumed, as exemplified in the preceding paper, is a challenge to nutritional investigators to uncover some dietary expedient by which this efficiency can be raised. Even though the evidence adduced in support of a not uncommon belief that a considerable proportion of the American people are subsisting upon borderline or inadequate levels of dietary calcium is not compelling, the practical usefulness in special and emer-

gency situations of some method by which a greater proportion of food calcium than the usual 20 to 40% can be put at the disposal of the tissues, is obvious. The present report is the first attempt of the authors to find such a method and while the results are not encouraging, they seem worth reporting. Some of their implications with reference to certain phases of calcium metabolism seem clear.

EXPERIMENTAL METHODS

The experiments were carried out upon seventeen of the nineteen subjects described in the preceding article. The technic of feeding, collecting of excreta, and chemical analysis was the same as previously described, except as the special purposes of these studies imposed special departures. The metabolism periods were 4 days in length and, on any one dietary regime, there were usually five such consecutive periods. The basal low-calcium diets were practically the same as those used in the earlier experiments. The effect of citric acid, sodium citrate, potassium citrate and orange juice upon the assimilation of the calcium in the basal diet and in the basal diet plus milk was studied, as well as the residual effects of these supplements in periods subsequent to their discontinuance. Some information was obtained also on the utilization of the calcium in calcium citrate as compared with that of calcium gluconate. The calcium in the latter compound has been shown to be as well utilized as that of milk (Steggerda and Mitchell, '39).

EXPERIMENTAL RESULTS

The tests of the utilization of calcium citrate and of calcium gluconate yielded the data presented in table 1. In this table will be found the excess calcium consumed over that contained in the basal low-calcium diet, together with the improvement in calcium balance brought about by the calcium salt supplement, both expressed in milligrams per day. These data are averaged for each salt, and the utilization computed by dividing the improvement in balance by the increase in

intake and multiplying by 100. The average utilization is 30 for the citrate salt and 25 for the gluconate. The difference cannot be considered significant because of the fewness of the data and their variability.

In table 2 are assembled the data secured when potassium citrate, 9 gm daily, or a mixture of potassium citrate, 4.5 gm daily, and citric acid, 4.5 gm daily, was added to the basal low-calcium diet to see whether these supplements would alter the output of dietary calcium at this low level, or would

TABLE 1

The utilization of the calcium in the citrate and gluconate salts. Average daily data.

SUBJECT	LENGTH OF TEST	EXCESS OVER BASAL		UTILIZATION
		Intake	Balance	
	days	*mg*	*mg*	%
		Calcium citrate		
Sn	20	+ 301	+ 69	
Sn	20	+ 326	+ 148	
To	20	+ 152	+ 21	
Hal	20	+ 326	+ 112	
Lo	20	+ 195	+ 39	
Averages		+ 260	+ 78	30
		Calcium gluconate		
Sn	20	+ 299	+ 19	
Sn	20	+ 360	+ 89	
Hal	20	+ 360	+ 103	
Lo	20	+ 171	+ 90	
Averages		+ 297	+ 75	25

change the balance of calcium. However, no consistent effect of the citrate ion is revealed by these studies. For the three subjects receiving the combination, the calcium balance averaged — 104 mg daily before the addition, and — 101 mg daily after the addition. For the two subjects receiving the potassium citrate supplement alone, the balances averaged — 124 mg before dosage, and — 128 mg during the 20-day dosage period.

When either sodium citrate or citric acid was added to milk consumed in such amounts as would induce slight negative balances of calcium, or at most very slight positive balances, the utilization of calcium was not appreciably or consistently affected. The evidence for this statement will be found in table 3. The average utilization percentages for milk alone and for milk plus sodium citrate for nine subjects averaged

TABLE 2

The effect of potassium citrate and citric acid on the utilization of calcium in the basal diet.

Results expressed in milligrams per day per 20-day period.

SUBJECT	SUPPLEMENTS TO BASAL DIET	CALCIUM METABOLISM			
		Intake	Feces	Urine	Balance
Steg	None	216	222	106	— 112
	Potassium citrate [1]	209	231	60	— 82
	Potassium citrate plus citric acid [2]	207	249	65	— 107
Step	None	213	267	83	— 137
	Potassium citrate [1]	216	276	56	— 116
	Potassium citrate plus citric acid [2]	222	279	93	— 150
Has	None	204	190	78	— 64
	Potassium citrate plus citric acid [2]	211	223	92	— 104

[1] 9 gm daily in three equal portions at meals.
[2] 4.5 gm of each daily in three equal portions at meals.

the same, namely, 32. For four subjects receiving milk alone and citric acid milk in adjacent periods, the percentage utilization also averaged the same, 25.

Although the citrate ion, in the amounts used, does not appear to modify appreciably the disposal within the adult human body of moderate levels of dietary calcium, throughout a 20-day period, when the dosage is discontinued the metabolism in subsequent periods may be markedly disturbed, as the data in table 4 demonstrate. In the 40- to 56-day interim between the first and the second citrate period, the calcium metabolism of subject Ro was not greatly affected, except

for a tendency for fecal calcium to decrease and for urinary calcium to increase at a somewhat greater rate. But for subjects Te, Steg, and Be, the balances became definitely less negative or more positive, generally due to a decreasing output of calcium, following an initial increase for two of the three men. Of particular interest is the large out-pouring of

TABLE 3

Utilization of the calcium of milk consumed alone, and, in an adjacent period, with sodium citrate or citric acid, 20-day periods except as indicated.

SUBJECT	MILK ALONE	MILK PLUS SODIUM CITRATE [1]	MILK ALONE	MILK PLUS CITRIC ACID [2]
	%	%	%	%
Steg	22	31	..	..
Steg	8	23	..	..
Steg	..	..	17 [3]	19
Ve	20	36	..	..
Se	36	26	..	..
Se	67	35	..	..
MeB	44	39	..	..
Be	0	28	..	..
Ro	29	31	..	..
Te	58	35	..	..
Ne	..	..	18	27
Cl	..	..	40	29
Fo	..	..	27 [3]	27
Averages	32	32	25	25

[1] 9 gm sodium citrate daily, given in capsules distributed evenly among the 3 meals.

[2] 0.27% citric acid in the milk.

[3] 16-day period.

calcium in the feces in the last low-calcium period for each of the four subjects, following the second citrate-feeding period. Comparing the final low-calcium period with the initial low-calcium period in this sequence of experiments for each subject, the daily fecal calcium increased 56, 89, 76, and 77%, for the four subjects in the order in which they are listed in the table. In assessing the significance of these increases, it should be remembered that the feces of successive 4-day periods were marked off with carmine, and that the data for

TABLE 4

The effect of sodium citrate on calcium metabolism in periods subsequent to its administration.

SUPPLEMENTS TO LOW-CALCIUM BASAL DIET	LENGTH OF PERIOD	DAILY CALCIUM METABOLISM				INDICATED UTILIZATION [1]
		Intake	Feces	Urine	Balance	
	days	*mg*	*mg*	*mg*	*mg*	%
Subject Ro						
None	16	201	232	177	— 208	
Milk + Na citrate	20	516	443	184	— 111	31
Milk	20	504	430	195	— 121	29
Milk	20	484	412	256	— 184	8
Milk	16	494	349	254	— 109	34
Milk + Na citrate	16	498	415	210	— 127	27
None	16	158	362	185	— 389	
Subject Te						
None	20	216	307	76	— 167	
Milk + Na citrate	20	498	467	98	— 67	35
Milk	20	517	444	65	+ 7	58
Milk	20	496	424	78	— 6	58
Milk + Na citrate	16	492	310	73	+ 109	100
None	6	264	581	54	— 371	
Subject Steg						
None	16	219	208	81	— 70	
Milk + Na citrate	20	518	390	129	— 1	23
Milk	20	493	445	96	— 49	8
Milk	20	517	352	101	+ 64	45
Milk	16	524	362	83	+ 79	49
Milk + Na citrate	16	494	324	86	+ 84	56
None	15	192	366	72	— 246	
Subject Be						
None	20	213	274	65	— 126	
Milk + Na citrate	20	532	466	103	— 36	28
Milk	20	535	534	128	— 127	0
Milk	20	502	408	93	+ 1	44
Milk	8	537	310	92	+ 135	81
Milk + Na citrate	16	512	396	75	+ 41	56
None	16	207	485	68	— 346	

[1] Computed on the basis of the first low-calcium period for each subject.

any 4-day period following a change in experimental treatment was disregarded if they were out of line with the data of the subsequent periods on the same diet. The calcium balances for all subjects were much more negative in the final low-calcium period than in the initial. The significance of these findings will be discussed later.

A daily supplement of 500 ml of orange juice (table 5) to the diets of four subjects, definitely depressed the utilization of milk calcium in subject Sw, an effect that persisted, apparently, for at least 40 days after the supplement was discontinued. In subjects Stég, Sn and To, the orange juice exerted little if any effect during the period of administration, but a more or less clear disturbing effect subsequently. This delayed effect was most pronounced with subject Sn, in whom the orange juice appeared to have induced a reduction in both fecal and urinary calcium extending over 72 days, so that the indicated utilization of milk calcium, computed on the basis of the first low-calcium period, was fictitiously high. This effect continued through a final low-calcium period. A similar reaction to orange juice, though not by any means so pronounced, was observed for subject To. The data for subject Steg extended over only one post-orange-juice period, but, such as they are, they indicated a depression of calcium utilization. For the three subjects for whom the sequence of experiments terminated in a low-calcium period, the calcium balance for the final period was much less negative, or in one case actually positive, as compared with the initial low-calcium period. This effect is just the opposite to that observed after citrate administration (table 4).

DISCUSSION

The experiments reported in this paper agree with most of the published evidence on normal animals and children in finding no effect of the citrate ion on calcium metabolism during a period of daily administration lasting 20 days. Also, calcium citrate does not seem to be clearly distinguished from

calcium gluconate or from milk in the utilization of its calcium by adult man.

However, after daily administration of citrates has ceased, profound effects upon calcium metabolism may ensue in subsequent periods. At approximately the same level of calcium intake, but especially on a low-calcium intake, a greatly increased output of calcium will generally occur, in feces or urine or both. Following this outpouring of calcium from the body, a reverse tendency may set in, to such an extent that the apparent utilization of calcium, computed on the basis of a low-calcium period just prior to citrate administration, may reach 80, or even 100%. However, for the entire sequence of experiments for each of the four subjects on this test (table 4), the total utilization of dietary calcium is not appreciably affected if consideration is given to the extra calcium excreted in the final, as compared with the initial, low-calcium period. If attention be restricted to the three subjects showing most distinctly the delayed effect of citrates above described, the uncorrected utilization percentages average 46, and the corrected percentages, 31.

These delayed effects of the citrate ion, extending over 40 to 60 days, can hardly relate to events occurring in the digestive tract, the contents of which are continually being removed and replaced from successive ingestions of food. Rather, they would seem to relate to metabolism and to cumulative effects of the citrate ion. The initial outpouring of calcium in the urine, but particularly in the feces, following a period of citrate administration, is in harmony with the observations of Gomori and Gulyas ('44), Lansing and Scott ('42), Shorr et al. ('42), and Marek, Wellmann and Urbányi ('42), except that these reports are concerned only with an increased output of calcium in the urine during a period of citrate dosage. The subsequent decrease in calcium output observed in our experiments, and in fact the entire picture of the delayed effect of the citrate ion on calcium metabolism, has no counter-part in anything we have found in the literature. It is hard to visualize on the basis of available informa-

tion the means by which these delayed effects are mediated. The most obvious inference is that during a period of citrate administration, citrate is stored in the body, later to be slowly released over a considerable period of time. Its effect on metabolism during the period of storage may be minimal if the storage tissues withdraw the citrate ion more rapidly from the blood stream than do the tissues subject to the metabolic influence of the ion. On discontinuance of the dosage, the storage tissues may release the ion for action elsewhere, the situation being somewhat analogous, perhaps, to the storage and release of the lead ion by the skeleton. In the case of citric acid, some 70% of the body's stores is said to be located in the skeleton (Dickens, '41), and these stores do not seem to be increased by citrate feeding (Leonards and Free, '44). These facts, if applicable to the adult human body, do not aid in explaining the delayed effects of the citrate ion on calcium metabolism.

The orange juice effect on calcium metabolism, illustrated by the data in table 5, is somewhat similar to the citrate effect for subjects Sn and To, but not for the other two men. In particular, there is no outpouring of calcium in the feces in the final low-calcium periods.

The observations reported in this paper bear upon the validity of a relatively new theory of calcium metabolism associated particularly with the names of McCance and Widdowson. Impressed by the fact that intravenous injections of calcium gluconate into normal subjects over a period of 2 weeks, in daily amounts equivalent to 186 mg of calcium, produced a rapid increase in urinary calcium but no detectable increase in fecal output of calcium, these investigators ('39) deduced the general conclusion "that the intestine does not normally excrete calcium . . . in amounts which are functions of plasma levels or metabolic requirements." This conclusion has been broadened still further into the belief that fecal calcium for all practical purposes consists entirely of calcium unabsorbed from that taken by mouth with the diet (McCance and Widdowson, '42). The fact, for example, that

TABLE 5

The effect of orange juice on calcium metabolism in periods subsequent to its administration.

SUPPLEMENTS TO LOW-CALCIUM BASAL DIET	LENGTH OF PERIOD	DAILY CALCIUM METABOLISM —				INDICATED UTILIZATION [1]
		Intake	Feces	Urine	Balance	
	days	*mg*	*mg*	*mg*	*mg*	*%*
Subject Steg						
None	20	167	231	58	— 122	
Milk	20	500	412	89	— 1	36
Milk + orange juice [2]	20	488	428	75	— 15	33
Milk	20	432	433	84	— 85	14
None	16	199	217	65	— 83	
Subject Sn						
None	20	261	280	182	— 201	
Milk	24	625	479	164	— 18	50
Milk + orange juice [2]	20	742	553	152	+ 37	49
Milk	20	657	468	129	+ 60	66
Milk	20	639	405	130	+ 104	81
Milk	12	664	523	127	+ 14	53
Chocolate milk [3]	20	632	430	117	+ 89	78
None	20	270	217	100	— 47	
Subject To						
None	36	192	86	210	— 104	
Milk	20	453	190	273	— 10	27
Milk + orange juice [2]	20	490	211	206	+ 73	43
Milk	20	418	134	186	+ 98	57
Chocolate milk [3]	20	396	153	155	+ 88	44
None	20	217	87	99	+ 31	
Subject Sw						
None	20	261	150	171	— 60	
Milk	24	371	197	191	— 17	40
Milk + orange juice [2]	20	481	309	220	— 48	5
Milk	20	401	247	277	— 123	— 45
Milk	24	386	250	262	— 126	— 52

[1] Computed on the basis of the first low-calcium period for each subject.

[2] 500 ml daily.

[3] In unpublished experiments on 8 adult human subjects, in cooperation with the Department of Home Economics, it was shown that chocolate in the amounts used in the preparation of chocolate milk, has no appreciable effect on the utilization of milk calcium.

an increase in protein intake of healthy adults will decrease the output of calcium in the feces and increase that in the urine has been interpreted to mean that amino acids resulting from protein digestion will increase the solubility of dietary calcium and thus its absorbability from the intestinal tract (McCance, Widdowson and Lehmann, '42). On the basis of such evidence, Hall and Lehmann ('44) have proposed the use of a calcium peptone powder to increase calcium absorption in hospital patients.

This broad interpretation of observations made under specific sets of experimental conditions is not justified and neglects entirely many observations of contrary significance made under other sets of experimental conditions. It disregards the well-established fact that the intestinal mucosa possesses more than a one-way permeability. Water and chlorides (Goldschmidt and Dayton, '19) and urea (Pendleton and West, '32) pass freely from the blood into the lumen of the alimentary tract under certain conditions. Bergeim ('26), by his method of detecting areas of absorption and areas of excretion in the tract, demonstrated clearly that, under certain conditions, calcium will pass from the blood to the interior of the intestine, an observation that has been confirmed by French and Cowgill ('37) and by Longwell in unpublished observations from this laboratory. In a more recent report, Greenberg ('45) has shown that, under his experimental conditions, 18.0 to 18.5% of an injected dose of radioactive calcium could be recovered from the feces. Possibly this effect is mediated through the bile (Greenberg and Troescher, '42).

The acidic or basic-character of the diet undoubtedly is one condition that determines to what extent absorbed calcium will be excreted through the intestinal walls and be voided with the feces. The reports of Zucker ('21) and of Bogert and Kirkpatrick ('22) show that acidic diets divert absorbed calcium to the urine, while alkaline diets divert absorbed calcium to the feces, often with little disturbance of the balance of calcium in the body. Farquharson, Salter and Aub

('31) showed that the tendency of a high-protein diet to divert absorbed calcium to the urine because of its acidic character could be obviated by simultaneous ingestion of $NaHCO_3$, both with a low-calcium and a moderate-calcium diet. In animals, such as the rabbit or the dairy cow, whose rations are habitually alkaline after oxidation, the urine contains very little calcium (Forbes et al., '22), but when given acid, the urinary output of calcium may increase enormously, while the fecal output decreases proportionately (Granström, '08).

The literature cited is only a sample of the evidence that could be marshalled against the contention that fecal calcium is unabsorbed calcium. It is true that much of this evidence is not conclusive, because it is subject to two interpretations, either the obvious one given to it in the above discussion, or the less probable one that the calcium in all potentially alkaline foods is less absorbable than that in potentially acid foods; or that acid ingested with the food always accelerates calcium absorption and that alkali depresses it. Some doubt has been cast upon the latter theory by Jones ('42).

The data contained in table 4, however, are unequivocal in their significance. Here, in four adult human subjects, the prior feeding of sodium citrate has, in the final low-calcium period, increased the fecal calcium output over that in the initial low-calcium period, by 56, 89, 76 and 77%, respectively, on generally lower intakes of calcium. In this final period, the fecal calcium exceeded the intake by 129, 120, 91, and 134%. Whether this represents a diversion of calcium from urine to feces by the alkalinity produced in the body on the oxidation of the acid radicle of sodium citrate, or some more specific effect of the citrate ion, cannot be decided. Unfortunately, the pH of the urines was not followed in these series of experiments. But regardless of the explanation, the data show indubitably that calcium is passing in large amounts from the blood stream to the lumen of the gastro-intestinal tract and that under these conditions, fecal calcium is obviously much more than unabsorbed food calcium.

CONCLUSIONS

1. Calcium citrate is approximately as well utilized by the adult human organism as is calcium gluconate.

2. Neither potassium citrate, nor a mixture of potassium citrate and citric acid, appreciably modifies the utilization of the calcium in a basal low-calcium diet.

3. The utilization in the adult organism of the calcium of milk is not appreciably or consistently modified by the simultaneous ingestion of sodium citrate, citric acid or orange juice in considerable amounts.

4. Sodium citrate and orange juice may induce profound disturbances in the calcium metabolism of adult men subsequent to a 20-day period of dosage, characterized generally by a phase of increased retention of calcium, followed, or preceded, or both, by a period of increased excretion. These changes are often beyond the limits of variation due to uncontrolled experimental factors and seem clearly traceable to the prior administration of the citrate ion.

5. Following citrate administration, the calcium in the feces of subjects subsisting on a low-calcium diet may amount to over twice the calcium intake. This is merely another instance, of many cited, in which fecal calcium contains much more than unabsorbed dietary calcium.

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LITERATURE CITED

BERGHEIM, O. 1926 Intestinal chemistry. VII. The absorption of calcium and phosphorus in the small and large intestines. J. Biol. Chem., vol. 70, p. 51.

BOGERT, L. J., AND E. E. KIRKPATRICK 1922 Studies in inorganic metabolism. II. The effects of acid-forming and base-forming diets upon calcium metabolism. J. Biol. Chem., vol. 54, p. 375.

CHANEY, M. S., AND K. BLUNT 1925 The effect of orange juice on the calcium, phosphorus, magnesium and nitrogen retention and urinary organic acid of growing children. J. Biol. Chem., vol. 66, p. 829.

CLASS, R. N., AND A. H. SMITH 1943 The skeleton as a source of endogenous citric acid. J. Biol. Chem., vol. 151, p. 363.

DAY, H. G., WITH THE TECHNICAL ASSISTANCE OF B. ECKENRODE 1940 The relation of phytin to the calcifying action of citrates. J. Nutrition, vol. 20, p. 157.

DICKENS, F. 1941 The citric acid content of animal tissues, with reference to its occurrence in bone and tumor. Biochem. J., vol. 35, p. 1011.

FARQUHARSON, R. F., W. T. SALTER AND J. C. AUB 1931 Studies of calcium and phosphorus metabolism. XIII. The effect of ingestion of phosphates on the excretion of calcium. J. Clin. Invest., vol. 10, p. 251.

FORBES, E. B., J. A. SCHULZ, C. H. HUNT, A. R. WINTER AND R. F. REMLER 1922 The mineral metabolism of the milch cow. J. Biol. Chem., vol. 52, p. 281.

FRENCH, R. B., AND G. R. COWGILL 1937 Immaturity of the organism as a factor determining the favorable influence of lactose on the utilization of calcium and phosphorus. J. Nutrition, vol. 14, p. 383.

GOLDSCHMIDT, S., AND A. B. DAYTON 1919 Studies in the mechanism of absorption from the intestine. I. The colon. A contribution to the one-sided permeability of the intestinal wall to chlorides. Am. J. Physiol., vol. 48, p. 419.

GOMORI, G., AND E. GULYAS 1944 Effect of parenterally administered citrate on the renal excretion of calcium. Proc. Soc. Exp. Biol. Med., vol. 56, p. 226.

GRANSTRÖM, E. 1908 Zur Frage über den Einfluss der Saüren auf Calciumstoffwechsel des Planzenfressers. Zeitsch. physiol. Chem., vol. 58, p. 195.

GREENBERG, D. M. 1945 Studies in mineral metabolism with the aid of artificial radioactive isotopes. VIII. Tracer experiments with radioactive calcium and strontium on the mechanism of vitamin D action in rachitic rats. J. Biol. Chem., vol. 157, p. 99.

GREENBERG, D. M., AND F. M. TROESCHER 1942 Study with radioactive isotopes of excretion of calcium and strontium by way of the bile. Proc. Soc. Exp. Biol. Med., vol. 49, p. 488.

HALL, T. C., AND H. LEHMANN 1944 Experiments on the practicability of increasing calcium absorption with protein derivatives. Biochem. J., vol. 38, p. 117.

HAMILTON, B., AND M. M. DEWAR 1937 Effect of citrate and tartrate on experimental rickets. Am. J. Dis. Child., vol. 54, p. 548.

HARTMANN, B. G., AND F. HILLIG 1934 Acid constituents of food products. Special reference to citric, malic and tartaric acids. J. Assn. Official Agric. Chemists, vol. 17, p. 522.

HATHAWAY, M. L., AND F. L. MEYER 1939 Citric acid and its salts as calcifying agents in rats. J. Nutrition, vol. 17, p. 419.

JONES, J. H. 1942 The relation of the pH of intestinal contents to calcium and phosphorus utilization. J. Biol. Chem., vol. 142, p. 557.

KETY, S. S. 1942 The lead citrate complex ion and its role in the physiology and therapy of lead poisoning. J. Biol. Chem., vol. 142, p. 181.

LANFORD, C. S. 1939 The effect of orange juice on calcium assimilation. J. Biol. Chem., vol. 130, p. 87.

———— 1942 a Effect on growth and calcium assimilation of citric acid-potassium citrate mixtures. J. Nutrition, vol. 23, p. 293.

———— 1942 b Studies of liberal citrus intakes. I. J. Nutrition, vol. 23, p. 409.

LANSING, A. I., AND G. H. SCOTT 1942 The effect of perfusion with sodium citrate on the content and distribution of the minerals in various cells of the cat as shown by electron microscopy and microincineration. Anat. Rec., vol. 84, p. 91.

LEONARDS, J. R., AND A. H. FREE 1944 The citrate content of the skeleton as influenced by prolonged feeding of acid-producing and base-producing salt. J. Biol. Chem., vol. 155, p. 503.

MCCANCE, R. A., AND E. M. WIDDOWSON 1939 The fate of calcium and magnesium after intravenous administration to normal persons. Biochem. J., vol. 33, p. 523.

———— 1942 Mineral metabolism of healthy adults on white and brown bread dietaries. J. Physiol., vol. 101, p. 44.

MCCANCE, R. A., E. M. WIDDOWSON AND H. LEHMANN 1942 The effect of protein intake on the absorption of calcium and magnesium. Biochem. J., vol. 36, p. 686.

MALLON, M. G., AND D. J. LORD 1942 Effect of lemon juice on calcium retention. J. Am. Dietet. Assn., vol. 18, p. 303.

MAREK, J., O. WELLMANN AND L. URBÁNYI 1942 Untersuchungen über den Mineral-stoffwechsel des tierischen Organisms. VI. Wirkung einer Dauereinfuhrung von Natriumcitratlösung auf den Calcium- und Phosphorstoffwechsel der Kuh und des Kaninchens. Arch. wiss. prakt. Tierheilk., vol. 77, p. 300.

METCALF, E. R., AND M. L. HATHAWAY 1945 Citrate metabolism of pre-school children. J. Nutrition, vol. 29, p. 211.

PENDLETON, W. R., AND F. E. WEST 1932 The passage of urea between the blood and the lumen of the small intestine. Am. J. Physiol., vol. 101, p. 391.

SHOHL, A. T. 1937 The effect of the acid-base content of the diet upon the production and cure of rickets with special reference to citrates. J. Nutrition, vol. 14, p. 69.

SHOHL, A. T., AND A. M. BUTLER 1939 Citrates in the treatment of infantile rickets. New England J. Med., vol. 220, p. 515.

SHORR, E., T. P. ALMY, M. H. SLOAN, H. TAUSSKY AND V. TOSCANI 1942 The relation between the urinary excretion of citric acid and calcium: its implications for urinary calcium stone formation. Science, vol. 96, p. 587.

STEGGERDA, F. R., AND H. H. MITCHELL 1939 The calcium requirement of adult man and the utilization of the calcium in milk and in calcium gluconate. J. Nutrition, vol. 17, p. 253.

WATSON, E. K., E. W. MCGUIRE, F. L. MEYER AND M. L. HATHAWAY 1945 Calcium metabolism of pre-school children. J. Nutrition, vol. 30, p. 259.

ZUCKER, T. F. 1921 The relation of acid base equilibrium in the body to excretion of phosphorus and calcium. Proc. Soc. Exp. Biol. Med., vol. 18, p. 272.

DENTAL CARIES IN THE COTTON RAT

VI. THE EFFECT OF THE AMOUNT OF PROTEIN, FAT AND CARBOHYDRATE IN THE DIET ON THE INCIDENCE AND EXTENT OF CARIOUS LESIONS [1]

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A high incidence and extent of carious lesions occurred in the molars of cotton rats fed diets high in sucrose or other soluble carbohydrates (Shaw et al., '44, '44 a; Schweigert et al., '45, '45 a; Shaw et al., '45). A low incidence and extent were noted when sufficient fluorine was added to the sucrose control ration, or when dextrin and stock rations were fed. This work has been extended to a study of the effect of the amount of fat, protein and carbohydrate in the ration on the incidence and extent of the lesions. The effect of feeding milk diets has also been studied. The results of these experiments are reported in this paper.

EXPERIMENTAL

The procedures described by Schweigert et al. ('45) have been followed in weaning the animals, dividing litter mates between groups on experiment, and calculating the growth

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rates. The method of evaluating the incidence and extent of the carious lesions after the 14-week experimental period has been reported by Shaw et al. ('44, '44 a).

The composition of the sucrose control ration 802, medium and high fat diets (821 and 814), high protein-medium fat diet (822) and high protein ration (813) is given in table 1. Four per cent of 1:20 liver extract was added to all diets at the expense of the entire ration and adequate quantities of the B vitamins were provided (McIntire, Schweigert and Elvehjem, '44). Each rat received 1 drop of halibut liver oil per week. In rations 814, 821, and 822, the lard was added isocalorically at the expense of the sucrose, thus maintaining the same mineral-vitamin-energy relation.

Whole liquid milk (fortified with iron, copper and manganese [3]) was fed to cotton rats as the sole source of food and water. 1:20 liver extract has been shown to increase the growth rate when added to purified rations (McIntire et al., '44; Schweigert et al., '45) without altering the incidence and extent of carious lesions (Shaw et al., '44). Therefore, for some groups, liver extract was homogenized into the milk at a level equivalent to 4% of the milk solids (0.5 gm of 1:20 liver extract per 100 ml of milk). A diet approximating the composition of milk solids (Rodgers, '35) was fed to compare the incidence and extent of tooth decay with those observed when liquid milk was fed. The composition of this ration (815) is given in table 1. Adequate quantities of the B vitamins (McIntire et al., '44) were provided and 4% of 1:20 liver extract was added at the expense of the entire ration.

RESULTS

The growth rates of cotton rats for the first 6 weeks and for 14 weeks on experiment and the incidence and extent of tooth decay are shown in table 2. A difference in the susceptibility to tooth decay between offspring from different parent stock has been noted in earlier work (Schweigert et al., '45 a);

[3] One mg of iron and 0.1 mg of copper and manganese were supplied daily to each animal.

therefore, the incidence and extent of dental caries observed for each experimental group and also for the litter mate controls fed the sucrose basal ration are presented in table 2.

The diets containing lard supported good growth, equal to that observed when the basal ration was fed. The growth rates when high protein diets were fed, also were similar to those observed for rats receiving the sucrose control ration. The growth rate observed when the rats were fed only mineralized whole milk was inferior to that observed when

TABLE 1

Composition of rations.
(The amount of each constituent is expressed in terms of parts of the ration.)

RATION	802 (SUCROSE BASAL)	913 (HIGH PROTEIN)	814 (HIGH FAT)	815 (MILK SOLIDS)	821 (MEDIUM FAT)	822 (HIGH PROTEIN-MEDIUM FAT)
Constituent						
Sucrose	67	41	22		44.5	18.5
Salts IV	4	4	4	5.4	4	4
Casein	24	50	24	27.3	24	50
Corn Oil	5	5	5		5	5
Lard			20		10	10
Lactose				39		
Butterfat				28.3		
Percentage of fat in the protein, carbohydrate and fat portion of the diet	5.2	5.2	35	30	18	18

the control ration was fed. The addition of 4% of 1:20 liver extract to the milk increased the growth rate approximately 2 gm per week for the first 6 weeks; however, the growth rate was still not as rapid as when the control ration was fed. The milk solids ration (815) supported better growth than whole milk but was inferior to the control diet. Apparently the cotton rat cannot tolerate high levels of lactose in the diet since the growth rate was retarded and a high mortality occurred when such rations were fed (table 2 and unpublished

TABLE 2

Effect of the level of fat and protein and milk diets on the incidence and extent of carious lesions.

RATION	NUMBER OF ANIMALS	GM GAINED/WEEK		AVE. INCIDENCE OF CARIOUS LESIONS	AVE. EXTENT OF CARIOUS LESIONS
		6 weeks	14 weeks		
802 + 4% 1 : 20 L.E.	3	9.3	7.1	33	113 +
814 (high fat) + 4% 1 : 20 L.E.	4	10.1	7.1	6	7 +
802 + 4% 1 : 20 L.E.	3	10.0	7.0	31	91 +
814 (high fat) + 4% 1 : 20 L.E.	4	9.0	6.1	3	4 +
821 (medium fat) + 4% 1 : 20 L.E.	4	9.2	6.9	13	24 +
802 + 4% 1 : 20 L.E.	4	10.5	7.6	28	78 +
821 (medium fat) + 4% 1 : 20 L.E.	4	11.1	8.1	13	29 +
802 + 4% 1 : 20 L.E.	3	9.3	7.1	33	113 +
813 (high protein) + 4% 1 : 20 L.E.	2	7.5	8.9	21	37 +
802 + 4% 1 : 20 L.E.	4	10.3	8.2	24	69 +
813 (high protein) + 4% 1 : 20 L.E.	5	8.8	6.0	11	24 +
822 (high protein-medium fat) + 4% 1 : 20 L.E.	5	9.7	7.1	1	1 +
802 + 4% 1 : 20 L.E.	3	10.6	6.1	23	67 +
Mineralized whole milk	5	5.0	4.5	0	0
Mineralized whole milk + 0.5 gm 1 : 20 L.E./100 ml	4	6.9	5.0	0	0
802 + 4% 1 : 20 L.E.	3	10.2	6.8	32	104 +
Mineralized whole milk + 0.5 gm 1 : 20 L.E./100 ml	3	8.3	5.3	0	0
802 + 4% 1 : 20 L.E.	3	11.1	8.5	20	58 +
815 (milk solids) + 4% 1 : 20 L.E.	3	8.1	6.6	5	7 +
802 + 4% 1 : 20 L.E.	6	8.9	7.2	32	108 +
815 (milk solids) + 4% 1 : 20 L.E.	3[1]	6.4	6.3	2	6 +

[1] Six animals were started in this group, but 3 failed to survive the 14-week experiment.

data). This fact has limited the amount of work that could be done with high lactose diets.

The ingestion of diets containing additional fat (814 and 821) resulted in a marked reduction in the caries occurrence. When 20 parts of lard were substituted at the expense of the sucrose, the average incidence and extent were 6 and 7 + and 3 and 4 + for the two groups, respectively. These figures are in contrast to the average incidence and extent of 33 and 113 + and 31 and 91 +, respectively, which were observed when the sucrose ration was fed to their litter mate controls. An intermediate caries incidence and extent were noted, 13 and 24 + and 13 and 29 +, respectively, when 10 parts of lard were substituted for sucrose in ration 821. Some reduction in the carious occurrence was noted when a 50% casein diet was fed (813), but when ration 822 was fed (10 parts of lard and 50 parts of casein) a further reduction in tooth decay was observed. The average incidence and extent of the lesions for the two groups when ration 813 was fed were 21 and 37 + and 11 and 24 +, respectively (table 2). When ration 822 was ingested an incidence of 1 and an extent of 1 + were obtained.

No cavities were observed in twelve animals that had been fed mineralized whole milk or mineralized whole milk plus 1:20 liver extract (table 2). In previous work when dextrin or stock rations were fed, a low incidence and extent were observed, but the protection was not as complete as when milk was fed. A low incidence and extent of tooth decay were noted when the milk solids ration (815) was fed. These results were comparable to those observed when the high fat ration 814 was fed.

DISCUSSION

These data show some of the interrelationships between the level and kind of carbohydrate, the amount of protein and fat in the diet and the incidence and extent of carious lesions in the cotton rat.

It has been found that partial caries protection was afforded when 50 parts of dextrin and 17 parts of sucrose were fed

[1] Six animals were started in this group, but 3 failed to survive the 14-week experiment.

in place of 67 parts of sucrose (Schweigert et al., '45). When dextrin was fed as the sole carbohydrate, a very low incidence and extent of lesions were noted. However, the ingestion of ration 821 (44.5 parts of sucrose and 10 parts of lard) resulted in a greater protection than when the above dextrin-sucrose ration was fed. In fact, when ration 814 was fed (20 parts of lard and 22 parts of sucrose) almost complete protection was observed, in spite of the fact that this ration contained more sucrose than the dextrin-sucrose diet (1.42 times as much on a percentage basis). Therefore, the effect of fat appeared to be more pronounced than that of dextrin in reducing the caries incidence.

The amount of tooth decay observed when ration 813 was fed (50 parts casein and 41 parts sucrose) was much lower than when the sucrose control ration was fed. The added protein in this ration (26 parts) appeared to produce about the same reduction in the number and extent of cavities as the substitution of 10 parts of lard to the diet (ration 821) and a greater reduction than the substitution of 50 parts of the sucrose with dextrin. The additive effect of 50 parts of casein and 10 parts of lard in the diet (18.5 parts of sucrose) was observed when the results were compared with those on high protein or 10 parts of lard in the diet. The protection was comparable to that observed when 20 parts of lard and 22 parts of sucrose were fed in ration 814.

These results help to explain why milk afforded complete protection against dental caries in the cotton rat. Milk on a solids basis is a high fat ration and the ingestion of fat has been shown to markedly reduce the caries occurrence. The presence of lactose as the sole carbohydrate in milk and a slightly higher level of protein may also have contributed to the low caries incidence observed.

Becks and coworkers ('44) have shown that a marked reduction in the frequency of dental caries in human subjects occurred when the intake of refined carbohydrates was reduced by replacement of the carbohydrates with meat, eggs, milk and milk products. The latter foods would not only

supply a small amount of carbohydrate, but would increase the fat and good quality protein levels of the diet. Jay et al. ('36) and Koehne et al. ('34, '34 a) have noted that the incidence of caries in humans was increased when sugar or "sweet foods" were ingested in appreciable amounts. Read and Knowles ('38) observed that in a caries-free group of children, the intake of fat and protein was good while in a caries-susceptible group the protein and fat intakes were deficient with the amount of carbohydrate consumed consequently increased. Boyd ('44) observed that the caries experience in 2 groups of children was comparably reduced even though the fat content of the diets differed by almost 100%. Therefore, he attributed the low number of cavities observed to the high nutritive value of the 2 diets and not to the low carbohydrate, high fat composition. The ratio of protein : carbohydrate : fat in the high and low fat diets was 7 : 9 : 21 and 7 : 15 : 11, and the percentage of fat was 57 and 33, respectively. In the present work fat comprised 35 and 18% of the protein, carbohydrate and fat portion of the high and medium fat diets, respectively (table 1). Therefore, the fat content of Boyd's lower fat diet was roughly equivalent to the fat content of the high fat diet used in these experiments. The amount of fat in his diets may be sufficiently high so that no difference in caries activity due to the fat content of the 2 diets could be noted. In earlier work Boyd et al. ('29) and Drain and Boyd ('30) offered additional evidence in support of the value of diets of high nutritive quality for reducing the caries experience in humans. Special diets were devised for four cases with celiac disease, with protein and dextrose supplying the energy (the latter as much as 60% of the total calories over a period of months) and with special emphasis on avoidance of fat and starch. No new caries were noted and all active caries were arrested by this dietary regimen in less than 10 weeks of dietary supervision.

The data obtained with the cotton rat indicate that high levels of fat and protein do not reduce the caries incidence by merely reducing the sucrose content of the diet, since con-

siderably higher amounts of sucrose are present in these diets than in dextrin-sucrose diets where a high caries incidence occurred.

Fat and protein are probably not as favorable for rapid fermentation by the acid-producing organisms associated with tooth decay processes. The dominating types of organisms in the oral cavity may be quite different when higher proportions of fat and protein are fed at the expense of sucrose in the ration. The effect of fat may be "physical" in that it forms a protective coating over the areas of the teeth most susceptible to invasion by the bacteria present in the mouth. A study of the effect of other dietary proteins and fats, in combination with several carbohydrates is needed to determine the complete interrelationship of the effects of the amount and kind of carbohydrate, protein and fat on the incidence and extent of dental caries in the cotton rat.

SUMMARY

1. The isocaloric substitution of 10 or 20 parts of lard for sucrose in a purified ration reduced the incidence and extent of carious lesions in the cotton rat in proportion to the amount of lard added.

2. When the casein content of the diet was increased from 24 to 50% at the expense of sucrose, some reduction in caries occurrence was observed.

3. When 50 parts of casein and 10 parts of lard were fed, the protective effect was additive. The number of cavities observed was comparable to that observed when the ration contained 20 parts of lard.

4. No carious lesions were noted when mineralized whole milk diets were fed. The incidence and extent of tooth decay were low when a ration approximating milk solids in composition was fed.

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LITERATURE CITED

BECKS, H., A. L. JENSEN AND C. B. MILLARR 1944 Rampant dental caries. Prevention and prognosis: A five year clinical survey. J. Dental Res., vol. 23, p. 210.

BOYD, J. D. 1944 Dental caries as influenced by fat versus carbohydrate in the diet. Am. J. Dis. Child., vol. 67, p. 278.

BOYD, J. D., C. S. DRAIN AND M. NELSON 1929 Dietary control of dental caries. Am. J. Dis. Child., vol. 38, p. 721.

DRAIN, C. S., AND J. D. BOYD 1930 Dietary control of dental caries. J. Am. Dent. Assn., vol. 17, p. 738.

JAY, P., F. P. HADLEY AND R. W. BUNTING 1936 Observation on the relationship of Lactobacillus acidophilus to dental caries in children during the experimental feeding of candy. J. Am. Diet. Assn., vol. 23, p. 846.

KOEHNE, M., AND R. W. BUNTING 1934 Studies on the control of dental caries. J. Nutrition, vol. 7, p. 657.

KOEHNE, M., R. W. BUNTING AND E. MORRELL 1934 Control of dental caries in children. Am. J. Dis. Child., vol. 48, p. 6.

MCINTIRE, J. M., B. S. SCHWEIGERT AND C. A. ELVEHJEM 1944 The nutrition of the cotton rat. (Sigmondon hispidus hispidus.) J. Nutrition, vol. 27, p. 1.

READ, T. T., AND E. M. KNOWLES 1938 Diet and habits of school children in relation to dental caries. British Dental J., vol. 64, p. 185.

RODGERS, L. A. 1935 Fundamentals of Dairy Science. 2nd Edition, p. 19. Reinhold Publishing Corporation.

SCHWEIGERT, B. S., J. H. SHAW, C. A. ELVEHJEM AND P. H. PHILLIPS 1945 a Dental caries in the cotton rat. V. Influence of strain variation on the caries susceptibility. Proc. Soc. Exp. Biol. Med., vol. 59, p. 44.

SCHWEIGERT, B. S., J. H. SHAW, P. H. PHILLIPS AND C. A. ELVEHJEM 1945 Dental caries in the cotton rat. III. Effect of different dietary carbohydrates on the incidence and extent of dental caries. J. Nutrition, vol. 29, p. 405.

SHAW, J. H., B. S. SCHWEIGERT, C. A. ELVEHJEM AND P. H. PHILLIPS 1944 a Dental caries in the cotton rat. II. Production and description of the carious lesions. J. Dental Res., vol. 23, p. 417.

SHAW, J. H., B. S. SCHWEIGERT, J. M. MCINTIRE, C. A. ELVEHJEM AND P. H. PHILLIPS 1944 Dental caries in the cotton rat. I. Methods of study and preliminary nutritional experiments. J. Nutrition, vol. 28, p. 333.

SHAW, J. H., B. S. SCHWEIGERT, P. H. PHILLIPS AND C. A. ELVEHJEM 1945 Dental caries in the cotton rat. IV. Inhibitory effect of fluorine additions to the ration. Proc. Soc. Exp. Biol. Med., vol. 59, p. 89.

THE INFLUENCE OF AUTOCLAVING SOYBEAN OIL MEAL ON THE AVAILABILITY OF CYSTINE AND METHIONINE FOR THE CHICK [1]

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It has been recognized for several years that cooking soybeans increases their protein nutritive value (Osborne and Mendel, '17; Hayward, Steenbock, and Bohstedt, '36; Hayward, '37). Hayward and Hafner ('41) and Almquist, Mecchi, Kratzer and Grau ('42) have demonstrated that the addition of dl-methionine to a diet containing raw soybean protein improved growth to a greater extent than when added to a diet containing the cooked soybean protein. The difference in the effectiveness of methionine observed by these investigators suggests that cooking the soybean oil meal increased the availability of cystine and methionine for the chick. Heat-treated soybean oil meal was shown by Almquist et al. ('42) to be slightly deficient in methionine for the chick when fed as the sole source of protein.

A wide variability in the nutritive value of the proteins of different commercial soybean oil meals was found by Draper and Evans ('44). The data obtained by Evans and St. John ('45) indicate that the differences in the availability of the proteins of the meals were caused by differences in extent of denaturation of the proteins by heat treatment. Hayward, Halpin, Holmes, Bohstedt and Hart ('37) found that heating at 140°C.

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for 2½ minutes or at 105°C. for 90 minutes produced a soybean oil meal with a higher protein nutritive value than did heating at 105°C. for 2 minutes or 82°C. for 90 minutes. Parsons ('43) obtained better growth of rats with a soybean oil meal autoclaved at 110°C. for 30 minutes than with one autoclaved at 123°C. for 30 minutes. Bird and Burkhardt ('43) obtained better chick growth with a soybean oil meal autoclaved for 2½ minutes at 128°C. than with one autoclaved for longer or shorter times at that temperature.

Marvel, Carrick, Roberts and Hauge ('44, '45) concluded that soybean oil meals were deficient in choline as well as methionine for growing chicks and that choline and methionine acted interchangeably to supplement the diet they used.

The purpose of the present investigation was to determine the influence of autoclaving soybean oil meal at different temperatures on the growth of chicks and on the availability of the cystine and methionine of soybean oil meal for chicks.

EXPERIMENTAL

Solvent-extracted soybean flakes [2] were used in this study. These flakes were ground in a hammer mill and well mixed. The required amount of the raw soybean oil meal for each diet was weighed into enameled pans. Each sample was spread in the bottom of the pan to a depth of about 1 inch. The meals were autoclaved for 30 minutes at steam pressures required to give temperatures of 100°C., 110°C., 120°C., and 130°C. One sample was autoclaved at 130°C. for 60 minutes.

Each of the diets fed had the following composition: cerelose, 53.0 gm; soybean oil meal, 29.0 gm; dried brewer's yeast, 5.0 gm; gelatin, 5.0 gm; mineral mixture,[3] 5.0 gm; soybean oil, 2.5 gm; fortified fish oil, 0.5 gm; tocopherol concentrate, 10.0 mg; p-aminobenzoic acid,[4] 10.0 mg; nicotinic acid, 3.0 mg;

[2] Supplied by Spencer — Kellog and Sons, Inc., Buffalo, N. Y.

[3] The composition of the mineral mixture was: oyster shell, 1570 gm; $CaHPO_4 \cdot 2H_2O$, 1740 gm; K_2HPO_4, 840 gm; NaCl, 600 gm; $MgSO_4 \cdot 7H_2O$, 500 gm; $Fe_2(SO_4)_3 \cdot XH_2O$, 55 gm; $MnSO_4 \cdot 4H_2O$, 29 gm; KI, 3.3 gm; $CuSO_4 \cdot 5H_2O$, 1.5 gm; $ZnCl_2$, 1.0 gm; and $CoCl_2 \cdot 6H_2O$, 0.2 gm.

[4] The crystalline vitamins were supplied by Hoffman-LaRoche, Inc., Nutley, N. J.

2-methyl-1, 4-naphthoquinone, 1.0 mg; riboflavin, 1.0 mg; calcium pantothenate, 1.0 mg; thiamine, 0.5 mg; and pyridoxine, 0.5 mg. The diets contained 21% protein. The only differences in the diets fed the different groups were in the heat treatment of the soybean oil meal and the additions of choline and methionine [5] to some of the diets. Descriptions of the heat treatment and per cent choline and methionine added to each diet are given in table 1.

Seventeen groups of fifteen New Hampshire chicks each were used in the experiment and were kept in electrically heated battery brooders with wire floors. The chicks were weighed individually at weekly intervals, and at the same time feed consumption was recorded. The duration of the experiment was 4 weeks. In order to determine the efficiency of utilization of cystine and methionine the droppings were collected on glass plates during the third week of the experiment. A careful record of gain in body weight and feed consumption was also kept for the third week. The droppings were air-dried, weighed, ground in a Wiley Mill, and sampled for chemical analysis.

Total sulfur was determined on the diets fed and on the droppings by the method described by Evans and St. John ('44). Inorganic and organic sulfur were determined as described by Evans and Greaves ('37), and cystine and methionine by the differential oxidation procedure described by Evans ('45). The cystine and methionine method is based on the principle that cystine is oxidized to sulfate by concentrated HNO_3, but methionine is not. Methionine is calculated from the total sulfur minus the sulfur oxidized to sulfate by HNO_3. Cystine is calculated from the sulfur oxidized to sulfate by HNO_3 minus inorganic sulfur. Cystine sulfur plus methionine sulfur thus equals organic sulfur. The retention of organic sulfur, cystine, and methionine by the growing chick was calculated from the results of the chemical analysis, and the amounts of feed consumed and droppings excreted.

[5] The methionine was supplied by Dr. L. C. Norris, Cornell University, Ithaca, N. Y.

TABLE 1

The influence of heat treatment and additions of choline and methionine on the nutritive value of soybean proteins.

GROUP NO.[1]	HEAT TREATMENT OF SOYBEAN MEAL		ADDITIONS TO DIET		WEIGHT AT 4 WEEKS (Average)	GAIN IN WEIGHT AT 4 WEEKS (Average)	PROTEIN EFFICIENCY (GAIN/PROTEIN)
	Time	Temp.	Choline	Methionine			
	min	*°C.*	%	%	*gm*	*gm*	*gm*
1	None				98 ± 19	58	1.14
2	30	110			192 ± 36	154	1.66
3	30	130			146 ± 33	106	1.43
4	None		0.2		114 ± 32	76	1.24
5	30	100	0.2		212 ± 33	174	1.81
6	30	110	0.2		194 ± 33	156	1.76
7	30	120	0.2		202 ± 44	162	1.66
8	30	130	0.2		159 ± 42	120	1.57
9	60	130	0.2		108 ± 22	69	1.09
10	None			0.2	188 ± 41	149	1.71
11	30	110		0.2	266 ± 40	226	2.09
12	30	130		0.2	253 ± 42	215	1.90
13	None		0.2	0.2	176 ± 22	137	1.90
14	30	110	0.2	0.2	298 ± 49	260	2.19
15	30	130	0.2	0.2	261 ± 41	223	1.95
16[2]	Commercial meal				183 ± 49	145	1.71
17	Practical[3] diet				254 ± 26	214	1.81

[1] Each group contained 15 chicks.

[2] The soybean oil meal used in this diet was commercially prepared.

[3] The practical diet was a commercial type diet such as would usually be fed growing chicks.

RESULTS

Total gain in body weight and gain per gram of protein consumed were used as criteria of the nutritive value of the proteins of the soybean oil meal and are presented in table 1. The raw soybean oil meal with no dietary supplement supported very poor growth in chicks. Autoclaving at 110°C. for 30 minutes increased the protein nutritive value of the soybean oil meal for chicks fed the basal diet alone or with added methionine, or methionine plus choline. Autoclaving at 130°C. increased the protein nutritive value, but not to as great an extent as autoclaving at 110°C.

When 0.2% choline was added to the basal diet, the soybean oil meals which had been autoclaved at 100, 110, or 120°C. gave better growth than the raw soybean oil meal or the soybean oil meals that were autoclaved at 130°C. for 30 or 60 minutes. The detrimental effect of overheating soybean oil meal on growth of chicks is shown in the group receiving the meal autoclaved for 60 minutes at 130°C., which had poorer total gain and gain per gram of feed consumed than the group receiving the raw meal.

The methionine content of the unsupplemented diet was 0.35% and the cystine plus methionine content was 0.63%. The addition of 0.2% dl-methionine to the diet raised the methionine content to 0.55% and the cystine plus methionine to 0.83%. The addition of 0.2% methionine in all cases greatly increased the body weights and the protein efficiency of chicks at 4 weeks over the groups receiving no added methionine. This level of methionine increased the total gain and gain per gram of protein consumed of chicks receiving raw soybean oil meal so that they equaled the values for the best heat-treated meal when unsupplemented with methionine. The addition of methionine to the soybean oil meal autoclaved at 130°C. for 30 minutes also increased its protein nutritive value so that it equaled that of the unsupplemented soybean oil meal autoclaved for 30 minutes at 110°C. The growth responses obtained by autoclaving the soybean oil meal at 110°C. and by supplementing the raw meal with 0.2% methionine

were approximately equal. The growth responses obtained separately by autoclaving at 110°C. and by supplementing with methionine were approximately additive when these treatments were combined.

The availability of the cystine and methionine in the different diets was determined by a sulfur balance study. The organic sulfur balance data are presented in table 2. Retention of organic sulfur is presented as grams retained and as per cent retained of the organic sulfur consumed, both for the total organic sulfur intake and the intake of organic sulfur from the basal diet. In calculating this latter value, it was assumed that all of the added dl-methionine was retained. This assumption appears logical since dl-methionine is fully utilized by the chick (Grau and Almquist, '43) and since no methionine appeared to be oxidized to sulfate. Further support of the assumption is that if such were not the case, the addition of methionine would appear to very greatly increase the methionine retention from the basal diet.

Chicks receiving soybean oil meal autoclaved at 100, 110, or 120°C. had a larger organic sulfur retention than chicks receiving the raw meal, whether supplemented with choline or methionine or receiving no supplements. Chicks receiving soybean oil meal autoclaved at 130°C. for 30 or 60 minutes retained a lower percentage of the organic sulfur than the chicks receiving soybean oil meal autoclaved at 100° or 110°C. for 30 minutes except that groups 12 and 15 (130°C.), which received added methionine, retained as high a percentage of the organic sulfur as groups 11 and 14 (110°C.).

The addition of 0.2% choline slightly increased the retention of organic sulfur in the group receiving raw soybean oil meal. The addition of 0.2% dl-methionine to the diets containing no added choline increased the percentage retention of the organic sulfur from the basal diets. Addition of choline plus methionine did not increase the retention of the organic sulfur of the diet.

There was a highly significant coefficient of correlation of +0.813 between the grams gain by chicks per gram of pro-

TABLE 2

Organic and inorganic sulfur balance of growing chick for 1-week period.

GROUP NO.	HEAT TREATMENT OF SOYBEAN MEAL		ADDITIONS TO DIET		ORGANIC S INTAKE FROM BASAL DIET	ORGANIC S INTAKE FROM ADDED METHIONINE	ORGANIC S EXCRETED	TOTAL ORGANIC S RETAINED		ORGANIC S FROM BASAL DIET RETAINED [1]		INORGANIC SULFUR		
	Time	Temp.	Choline	Methionine								Intake	In droppings	Balance
	min	*°C.*	%	%	*gm*	*gm*	*gm*	*gm*	%	*gm*	%	*gm*	*gm*	*gm*
1	None				1.28		0.85	0.43	34	0.43	34	0.90	0.74	+ 0.16
2	30	110			2.89		1.38	1.51	52	1.51	52	2.03	1.59	+ 0.44
3	30	130			2.04		1.20	0.84	41	0.84	41	1.43	1.24	+ 0.19
4	None		0.2		1.61		0.83	0.78	48	0.78	48	1.13	0.91	+ 0.22
5	30	100	0.2		3.22		1.32	1.90	59	1.90	59	2.27	1.76	+ 0.51
6	30	110	0.2		2.77		1.29	1.48	53	1.48	53	1.95	1.44	+ 0.51
7	30	120	0.2		3.07		1.31	1.76	57	1.76	57	2.16	1.54	+ 0.62
8	30	130	0.2		2.22		1.23	0.99	45	0.99	45	1.56	1.17	+ 0.39
9	60	130	0.2		1.55		1.00	0.55	35	0.55	35	1.09	0.80	+ 0.29
10	None			0.2	2.52	0.71	1.40	1.84	57	1.13	45	1.78	1.39	+ 0.39
11	30	110		0.2	3.66	1.04	1.49	3.21	68	2.17	59	2.58	1.87	+ 0.71
12	30	130		0.2	3.60	1.02	1.43	3.19	69	2.17	60	2.54	1.39	+ 1.15
13	None		0.2	0.2	2.58	0.73	1.64	1.68	51	0.94	36	1.82	1.43	+ 0.39
14	30	110	0.2	0.2	3.50	0.99	1.78	2.71	60	1.72	49	2.46	1.89	+ 0.57
15	30	130	0.2	0.2	3.74	1.06	1.97	2.83	59	1.77	47	2.63	1.96	+ 0.67
16	Commercial meal				2.78		1.33	1.45	52	1.45	52	1.94	1.57	+ 0.37
17	Practical diet				6.18		2.84	3.34	54	3.34	54	1.24	1.24	+ 0.00

[1] This assumes that all added dl-methionine is utilized and retained.

tein consumed (table 1) and the per cent of organic sulfur retained of that ingested by the chicks (table 2) for the seventeen diets. This highly significant correlation indicates that the availability of the organic sulfur limited the extent to which the protein was utilized by the growing chick under the conditions of this experiment.

The results of the cystine and methionine balance studies are presented in table 3. The cystine sulfur was very poorly retained. The soybean oil meals heated at 100°, 110°, or 120°C. gave a better retention of cystine than the raw meals or those heated at 130°C., whether supplemented with choline or receiving no supplements, except for groups 12 and 15. Retention of methionine sulfur was improved by heating the meals at 100°, 110°, or 120°C. Heating at 130°C. increased methionine retention but not as much as heating at 110°C. Addition of methionine to the diet containing no added choline increased methionine retention, but when added to the diet containing added choline no increase in methionine retention occurred.

There appeared to be some retention of inorganic sulfur, since every group had a larger intake of inorganic sulfur than was excreted (table 2).

DISCUSSION

According to Grau and Almquist ('43) the methionine requirement for growing chicks is 0.5 to 0.6% of the diet and the cystine plus methionine requirement is 1.0 to 1.1%. All diets used in this experiment except the practical diet were deficient in methionine plus cystine according to these standards. The diets to which no supplemental methionine was added were deficient in methionine. With the exception of the raw meal, the growth and protein efficiency were better on the diets to which methionine was added than on the practical diet which contained 0.46% methionine and 1.03% cystine plus methionine. It is not known whether the addition of methionine to the practical diet or the addition of cystine to the diets containing added methionine would have further increased growth and protein efficiency. The very good growth

TABLE 3

Cystine and methionine balance of growing chicks for 1-week period.

GROUP NO.	HEAT TREATMENT OF SOYBEAN MEAL		ADDITIONS TO DIET		METHIONINE S INTAKE FROM BASAL DIET	METHIONINE S INTAKE FROM ADDED METHIONINE	METHIONINE S EXCRETED	METHIONINE S RETAINED		METHIONINE S FROM BASAL DIET RETAINED [1]		CYSTINE S INTAKE FROM BASAL DIET	CYSTINE S EXCRETED	CYSTINE S RETAINED	
	Time	Temp.	Choline	Methionine											
	min	*°C.*	%	%	*gm*	*gm*	*gm*	*gm*	%	*gm*	%	*gm*	*gm*	*gm*	%
1	None				0.64		0.27	0.37	58	0.37	58	0.64	0.57	0.07	11
2	30	110			1.44		0.37	1.07	74	1.07	74	1.44	1.01	0.43	30
3	30	130			1.02		0.30	0.72	71	0.72	71	1.02	0.89	0.13	13
4	None		0.2		0.81		0.23	0.58	72	0.58	72	0.81	0.60	0.21	26
5	30	100	0.2		1.61		0.24	1.37	85	1.37	85	1.61	1.07	0.54	34
6	30	110	0.2		1.38		0.30	1.08	78	1.08	78	1.38	0.99	0.39	28
7	30	120	0.2		1.54		0.28	1.26	82	1.26	82	1.54	1.02	0.52	34
8	30	130	0.2		1.11		0.30	0.81	73	0.81	73	1.11	0.93	0.18	16
9	60	130	0.2		0.78		0.31	0.47	60	0.47	60	0.78	0.69	0.09	12
10	None			0.2	1.26	0.71	0.36	1.61	82	0.90	71	1.26	1.04	0.22	17
11	30	110		0.2	1.83	1.04	0.28	2.59	90	1.55	85	1.83	1.20	0.63	34
12	30	130		0.2	1.80	1.02	0.37	2.45	87	1.43	79	1.80	1.06	0.74	41
13	None		0.2	0.2	1.29	0.73	0.54	1.48	73	0.75	58	1.29	1.10	0.19	15
14	30	110	0.2	0.2	1.75	0.99	0.43	2.31	84	1.32	75	1.75	1.35	0.40	23
15	30	130	0.2	0.2	1.87	1.06	0.51	2.42	83	1.36	73	1.87	1.46	0.41	22
16	Commercial meal				1.39		0.40	0.99	71	0.99	71	1.39	0.92	0.47	34
17	Practical diet				2.43		0.83	1.60	66	1.60	66	3.74	2.01	1.73	46

[1] This assumes that all added dl-methionine is utilized and retained.

and protein efficiency of the chicks receiving the soybean oil meal that had been autoclaved at 110°C. and 0.2% supplemental methionine suggests the possibility of less than 1.0% cystine plus methionine being required by the chick when more than 0.5% is present as methionine. Methionine was much better utilized than cystine.

Choline and methionine did not exert an interchangeable supplementary action on the diet used. This does not agree with the findings of Marvel, Carrick, Roberts, and Hauge ('44) using a corn and soybean oil meal diet. Choline may have supplemented all of the diets to a slight extent, but methionine had a much greater supplementary action.

The protein nutritive value of raw soybean oil meal was greatly increased by autoclaving for 30 minutes at 100°, 110°, or 120°C., whether or not the diet contained added choline or methionine. Since the addition of 0.2% methionine to the diet containing raw soybean oil meal increased the protein nutritive value almost as much as did the best heat treatment, it would appear that autoclaving increased the availability of the cystine and methionine, as was believed by Hayward and Hafner ('41) and Almquist, Mecchi, Kratzer, and Grau ('42). This is further substantiated by the greater retention of cystine and methionine from the diets containing the moderately heated soybean oil meals (100–120°C.). Further heat treatment decreased the protein nutritive value of the soybean oil meal. This appeared to be caused by a decreased availability of the cystine and the methionine since the addition of methionine to the overheated meals increased growth and gain per gram of protein consumed to equal those of the soybean oil meal autoclaved for 30 minutes at 110°C. and since there was a poor retention of the dietary cystine and methionine on the diets containing the overheated soybean oil meals.

It is probable that the higher autoclaving temperature did not exert the harmful effect on the soybean oil meal simply by decreasing the availability of the methionine and cystine, but since the diet was deficient in methionine this amino acid was the limiting one in all cases. The decrease in protein

nutritive value was thus due to lowered methionine availability even though the availability of all amino acids may have been decreased. Evans and St. John ('45) showed a progressive denaturation of soybean proteins with increased heat treatment of the soybean oil meal. According to data presented in the present paper this denaturation was accompanied by a decreased availability of methionine. The decreased methionine availability may have been caused by a lowered availability of some of the denatured proteins. It appears that the decreased availability was not caused by a decreased digestibility according to data obtained by Johnson, Parsons, and Steenbock ('39) from sulfur balance studies with rats.

The methionine retention was much better than the cystine retention. Part of the poor cystine retention may have been exaggerated because of shedding of feathers, some of which became mixed with the droppings. Most of these, however, were separated before weighing and grinding the droppings.

It was expected that there would be considerable oxidation of cystine and methionine to sulfate but this was not the case, since there was less sulfate sulfur in the droppings than in the feed, indicating a retention of some of the sulfate sulfur. However, it is possible that some sulfate sulfur was lost from the droppings through bacteriological action.

Methionine may act as a source of methyl groups to replace choline (du Vigneaud, Cohn, Chandler, Schenck, and Simmonds, '41), so that in a deficiency of choline, methionine may supply methyl groups. To the extent that choline replaces methionine in this capacity, it probably replaces methionine in the diet.

SUMMARY

The nutritive value of the proteins of raw soybean oil meal, as determined by total gain in weight and gain per gram of protein consumed by chicks, was increased by autoclaving the meal at 100°C., 110 C., or 120°C. for 30 minutes. The protein nutritive values were lower when soybean oil meal was

autoclaved at 130°C. for 30 or 60 minutes than when it was autoclaved at 100°–120°C.

The availability for growing chicks of the methionine and cystine or the organic sulfur in soybean oil meal was increased by autoclaving the raw meal. The availability of the methionine and cystine or the organic sulfur was not as great when the meals were autoclaved at temperatures above 120°C. as it was when the meals were autoclaved at 100°C., 110°C., or 120°C. Added methionine increased the growth and feed efficiency on all diets. The addition of 0.2% dl-methionine to the basal diet increased the retention by the growing chick of the methionine originally in the unsupplemented diet. The methionine of the soybean oil meal was retained to a much greater extent than the cystine.

LITERATURE CITED

ALMQUIST, H. J., E. MECCHI, F. H. KRATZER AND C. R. GRAU 1942 Soybean protein as a source of amino acids for the chick. J. Nutrition, vol. 24, p. 385.

BIRD, H. R., AND G. J. BURKHARDT 1943 Factors affecting the nutritive value of soybean oil meals and soybeans for chickens. Maryland Agr. Exp. Sta., Bull. no. A27, p. 35.

DRAPER, C. I., AND R. J. EVANS 1944 Plant protein concentrates in the chick ration. Poultry Sci., vol. 23, p. 507.

DU VIGNEAUD, V., M. COHN, J. P. CHANDLER, J. R. SCHENCK AND S. SIMMONDS 1941 The utilization of the methyl group of methionine in the biological synthesis of choline and creatine. J. Biol. Chem., vol. 140, p. 625.

EVANS, R. J. 1945 Determination of the cystine and methionine content of plant and animal material by a differential oxidation procedure. Arch. Biochem., vol. 7, p. 439.

EVANS, R. J., AND J. E. GREAVES 1937 Some factors influencing the sulfur content of alfalfa. Proc. Utah Acad. Sci., vol. 14, p. 17.

EVANS, R. J., AND J. L. ST. JOHN 1944 Determination of total sulfur in feeds. Modified nitric and perchloric acid digestion procedure. Ind. Eng. Chem., Anal. Ed., vol. 16, p. 630.

———— 1945 Estimation of the relative nutritive value of vegetable proteins by two chemical methods. J. Nutrition, vol. 30, p. 209.

GRAU, C. R., AND H. J. ALMQUIST 1943 The utilization of the sulfur amino acids by the chick. J. Nutrition, vol. 26, p. 631.

HAYWARD, J. W. 1937 The nutritive value of soybean oil meal prepared by the different methods of oil extraction. Oil and Soap, vol. 14, p. 317.

HAYWARD, J. W., AND F. H. HAFNER 1941 The supplementary effect of cystine and methionine upon the protein of raw and cooked soybeans as determined with chicks and rats. Poultry Sci., vol. 20, p. 139.

HAYWARD, J. W., J. G. HALPIN, C. E. HOLMES, G. BOHSTEDT AND E. B. HART 1937 Soybean oil meal prepared at different temperatures as a feed for poultry. Poultry Sci., vol. 16, p. 3.

HAYWARD, J. W., H. STEENBOCK AND G. BOHSTEDT 1936 Effect of heat as used in the extraction of soybean oil upon the nutritive value of the protein of soybean oil meal. J. Nutrition, vol. 11, p. 219.

JOHNSON, L. M., H. T. PARSONS AND H. STEENBOCK 1939 Effect of heat and solvents on the nutritive value of soybean protein.. J. Nutrition, vol. 18, p. 423.

MARVEL, J. A., C. W. CARRICK, R. E. ROBERTS AND S. M. HAUGE 1944 The supplementary value of choline and methionine in a corn and soybean oil meal chick ration. Poultry Sci., vol. 23, p. 294.

———— 1945 The value of choline additions to a corn and soybean oil meal chick ration containing distillers' dried solubles. Poultry Sci., vol. 24, p. 181.

OSBORNE, T. B., AND L. B. MENDEL 1917 The use of soybean as a food. J. Biol. Chem., vol. 32, p. 369.

PARSONS, H. T. 1943 Effect of different cooking methods on soybean proteins. J. Home Economics, vol. 35, p. 211.

THE CAROTENE CONTENT OF CUBAN FOODS

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The carotene content of tropical plant and animal materials is not yet well known. The variety and exuberance of the flora and fauna of the tropics suggest the likely possibility of finding excellent sources of various members of the vitamin group including carotene. This is supported by the recent report of Cravioto, Lockart et al. ('45) on analyses of Mexican foods. These authors have recorded the exceptional nutritive value of some less commonly used foods such as the malvas for example. This report has encouraged us in our work of analysing Cuban foods, work which was begun long before the above mentioned report was published. In this paper we present the results of carotene analyses of seventy-five samples of forty-three Cuban foods, several of which, to the best of our knowledge, have never been analysed before.

METHODS AND MATERIALS

As chromatographic separation of the carotenes from pigmented impurities has been demonstrated to be more specific than the commonly used biphasic purification, we chose a technique of the first type, namely the method of Wall and Kelley ('43). Except in the case of cacao, their modification for fresh material was employed. The use of a regulator for controlling the speed of the Waring blendor was found necessary in many instances to avoid splashing and subsequent loss of carotene. This happened particularly in samples of soursop, canistel and cashew nut.

All of the samples were secured from the Havana curb markets, and were of the same quality and degree of ripeness as the food commonly consumed. Whenever possible the variety was recorded and two or three determinations of the same food were made. In the case of mango eight varieties were assayed. Only the edible part of the fruits was considered.

When extraction in the blendor resulted in white extracts the result was reported as carotene-free. It should be noted that a yellow extract sometimes contained non-measurable carotene, as coloring impurities had been retained by the chromatographic column; this was true for samples of peanut oil and grapefruit. When a final extract of a 100-gm sample was concentrated to 10 ml, and gave a photometric reading of more than a 91% transmission, the sample was also reported as carotene-free.

The malanga, mamoncillo and pitahaya samples were hard to filter through fritted glass while that of mango was the easiest. Filtration was accelerated when a funnel with a capacity that was several times the volume to be filtered was employed.

We used Kimble amber and Pyrex low actinic glasses, although these are not specified for carotene determinations. We found that both colored glasses, especially the Pyrex red, made it difficult to observe the emulsions that were frequently encountered.

RESULTS AND DISCUSSION

The results of our analyses are presented in table 1. Examination of the table reveals that the mango, mamey de Santo Domingo, canistel and red cashew nut are the Cuban grown fruits richest in carotene. Next to these come the muskmelon, tangerine, Johnson banana, guava, red mamey and Jamaica cherry. Fair values were found for red pepper, yellow malanga and ripe plantain. Except for the Placero tomato, the rest of the foods analysed were found to be unimportant with respect to content of carotene. Soursop and coconut

were completely devoid of the pro-vitamin, while peanut oil, prú and grapefruit contained only non-measurable amounts.

The most interesting finding was the remarkably high carotene content of certain varieties of mango, values which prove to be the highest ever recorded among fruits. Of the eight varieties assayed, the Bizcochuelo and the Mulgoba showed very high values, while the others had about the same content as has been found in other countries. In one sample of Moro mango 77% of its crude carotene was found to be beta-carotene. A single Moro, Bizcochuelo or Mulgoba mango contains several times the daily allowance for adults of vitamin A recommended by the Food and Nutrition Board of the U. S. National Research Council ('43). In this connection it is of some interest to mention that Valledor and Fernández Flores ('40) and Rosenkranz and Vieta ('45) have found the mango to be a good source of vitamin C as well.

A comparison of our figures with those of Booher, Hartzler and Hewston ('42) and of Cravioto et al. ('45) shows that Cuban fruits are, in general, richer in carotene than the corresponding American and Mexican fruits. The values for tomato, red pepper, lemon, grapefruit, honey, potato, cucumber, watermelon and banana agree with those found for the same foods grown and analysed in the United States, and similarly, in the case of red mamey, sugar cane, guava, fig, lime, lemon, and some mango varieties with those for these foods grown and assayed in Mexico. The only difference between the American assays and ours pertain to papaya, and between the Mexican data and ours with respect to the banana. Climate, botanical variety, variation among samples, and no doubt other factors may be responsible for this. Appreciable variation in carotene content among different samples was experienced in many cases, but this is of frequent occurrence in food analyses, especially when common market samples are used as was the case in our study. It should be pointed out that the particular method of analysis employed by us is highly specific.

TABLE 1

Carotene content of Cuban foods.

COMMON NAME	BOTANICAL NAME	VARIETY	CAROTENE
			mg/gm of material
Banana	Musa sapientum, Lin.	Johnson	2.4
Banana	Musa sapientum, Lin.	Manzano	0.3
Bee's honey			0.02
Cacao, raw	Theobroma cacao, Lin.	Baracoa	1.7
Cacao, toasted	Theobroma cacao, Lin.		2.1
Canistel	Lucuma nervosa, A.D.C.		9.5
Canistel	Lucuma nervosa, A.D.C.		74.0
Cashew nut	Anacardium occidentale, Lin.	Red	4.2
Cashew nut	Anacardium occidentale, Lin.	Red	9.5
Cashew nut	Anacardium occidentale, Lin.	Red	15.5
Coconut, flesh [1]	Cocos nucifera, Lin.		0.0
Coconut, flesh [1]	Cocos nucifera, Lin.		0.0
Coconut, milk [1]	Cocos nucifera, Lin.		0.0
Coconut, milk [1]	Cocos nucifera, Lin.		0.0
Cucumber	Cucumis sativus, Lin.		0.2
Chayote	Sechium edulis (Jacq.) S.W.	Spiny	0.03
Cherry, Jamaica	Malpighia glabra, Lin.		1.3
Fig	Ficus carica, Lin.		0.4
Fig	Ficus carica, Lin.		0.6
Grapefruit, juice [2]	Citrus decumana, Lin.		0.0
Grapefruit, juice [2]	Citrus decumana, Lin.		0.0
Guagüí	Colocasia antiquorum, Schott		0.2
Guava	Psidium guajaba, Lin.		1.6
Guava	Psidium guajaba, Lin.		2.8
Lemon, common	Citrus limonum, Risso		0.04
Lemon, sour (rind)	Citrus medica, Lin.		0.02
Lime, juice [2]	Citrus limetta, Risso		0.0
Lime, juice	Citrus limetta		0.02
Malanga [2]	Xanthosoma sagitifolium, Schott	White	0.0
Malanga	Xanthosoma sagitifolium, Schott	Yellow	10.8
Mamey, red	Achras zapota, Lin.		1.4
Mamey, red	Achras zapota, Lin.		2.4
Mamey de Sto. Domingo	Mammea americana, Lin.		11.7
Mamey de Sto. Domingo	Mammea americana, Lin.		14.0
Mamey de Sto. Domingo	Mammea americana, Lin.		52.0
Mamoncillo	Melicocca bijuga, Lin.		0.03
Mango	Mangifera, indica, Lin.	Bizcochuelo	41.5
Mango	Mangifera, indica, Lin.	Bizcochuelo	72.5
Mango	Mangifera, indica, Lin.	Filipino	9.5

TABLE 1 (*Continued*)

COMMON NAME	BOTANICAL NAME	VARIETY	CAROTENE
			mg/gm. of material
Mango	Mangifera, indica, Lin.	Julián	19.0
Mango	Mangifera, indica, Lin.	Manga amarilla	20.8
Mango	Mangifera, indica, Lin.	Mango de puerco	16.4
Mango	Mangifera, indica, Lin.	Moro	115.0
Mango	Mangifera, indica, Lin.	Moro	139.5
Mango	Mangifera, indica, Lin.	Moro	164.0
Mango	Mangifera, indica, Lin.	Mulgoba	51.6
Mango	Mangifera, indica, Lin.	Toledo	16.4
Muskmelon	Cucumis melo, Lin.		7.6
Okra	Hibiscus esculentus, Lin.		2.4
Orange, sour (juice)	Citrus aurantium, Lin.		0.3
Orange, sour (juice)	Citrus aurantium, Lin.		1.3
Papaya	Carica papaya, Lin.	Yellow	0.6
Peanut oil [3]			0.0
Pepper	Capsicum annum, Lin.	Common red	27.6
Pitahaya	Selenicereus grandiflorus (L.) B and R		0.05
Plantain, ripe	Musa paradisiaca, Lin.	Burro	9.2
Potato	Solanum tuberosum, Lin.		0.02
Prú [2]			0.0
Sapodilla	Sapota achras, Mill.		0.2
Sapodilla	Sapota achras, Mill.		1.2
Sirup, sugar cane			0.03
Sirup, sugar cane			0.04
Soursop [2]	Annona muricata, Lin.		0.0
Soursop [2]	Annona muricata, Lin.		0.0
Sugar cane	Saccharum officinarum, Lin.		0.2
Sweetpotato	Ipomoea batatas, Lin.	White	2.1
Tamarind	Tamarindus indicus, Lin.		0.3
Tamarind	Tamarindus indicus, Lin.		0.8
Tangerine, juice	Citrus nobilis, Lour.		7.5
Tomato	Lycopersicum esculentum, Will.	Placero	5.8
Watermelon	Citrullus citrullus (L.) Karst		0.5
Watermelon	Citrullus citrullus (L.) Karst		1.3
Watermelon	Citrullus citrullus (L.) Karst		1.3
Yam, white	Dioscorea alata, Lin.		0.03
Yam, yellow	Dioscorea cayenensis, Lin.		0.1

[1] A 100-gm sample rendered a white extract in the blendor.

[2] Final extract of a 100-gm sample concentrated to 10 ml gave a valueless photometer reading.

[3] A 10-ml sample gave the same results as in [2].

SUMMARY

The carotene content of seventy-five samples of forty-three Cuban plant foods was determined by the chromatographic technique of Wall and Kelley. Of the eight mango varieties assayed, three gave the highest values ever recorded among fruits. These values were higher than those for carrots and similar to the highest reported for spinach, both vegetables being analysed in the United States. Of the remaining mango varieties, several gave values similar to those reported from other countries. In one sample of the Moro variety 77% of the carotene proved to be beta-carotene. This variety had 115, 139.5 and 164 μg of carotene per gram of pulp, respectively, in three samples. In general Cuban fruits are richer in carotene than the American and Mexican grown varieties.

The mango, canistel, mamey de Santo Domingo and red cashew nut had the highest values among Cuban fruits. A listing of the remaining "good" foods in order of decreasing carotene content gives the following: red pepper, yellow malanga, ripe plantain, muskmelon, tangerine, Placero tomato, guava, red mamey, okra and Johnson banana. Other foods would be rated appreciably below these; and the soursop, coconut, prú, grapefruit and peanut oil either lack carotene or have it in non-measurable amounts.

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LITERATURE CITED

BOOHER, L., E. HARTZLER AND E. HEWSTON 1942 A compilation of the vitamin values of foods in relation to processing and other variants. U. S. Dept. Agric. Circular No. 638.

CRAVIOTO, R., E. LOCKART, R. ANDERSON, F. MIRANDA AND R. HARRIS 1945 Composition of typical Mexican foods. J. Nutrition, vol. 29, p. 317.

FOOD AND NUTRITION BOARD 1943 Recommended Dietary Allowances. National Research Council, Reprint and Circular Series No. 115.

ROSENKRANZ, G., AND E. VIETA 1945 Unpublished work.

VALLEDOR, T., AND E. FERNÁNDEZ FLORES 1940 Determinación de la vitamina C en las frutas de Cuba. Bol. Soc. Cubana de Pediat., vol. 12, p. 547.

WALL, M., AND E. KELLEY 1943 Determination of pure carotene in plant tissue. Ind. Eng. Chem., Anal. Ed., vol. 15, p. 18.

THE EFFECTS OF ADDED VITAMIN A ON THE CONJUNCTIVA AND THE LEVEL OF VITAMIN A IN THE BLOOD

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ONE FIGURE

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Kruse ('41) reported that 99% of 166 adults in a low income group showed diminished transparency of the bulbar conjunctiva which he claimed was due to avitaminosis A. In 39% of his subjects elevations or spots which could be seen with the naked eye were present. Following the administration of 100,000 I.U. of vitamin A daily for 8 months to 70 adults, Kruse reported the following results:

"One person with spots has been completely restored and discharged. In all others with spots, the conjuctiva has become less vascular, thinner, clearer and more lustrous. The spots are much diminished in size; in many no longer grossly elevated; in some detectible only by microscope. Of the persons with 'nonspot' lesions, eight have been fully restored and discharged. Naturally, since the 'nonspot' lesions are usually less severe, more in this group were among the first to show complete recovery. Nevertheless, they have required not less than 6 months' intensive therapy.

"In both groups those who have not received therapy have shown no improvement."

EXPERIMENTAL

In the following test, forty pupil nurses, who needed 1 to 2 years more to complete their training, agreed to act as sub-

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jects. Thirty-four of the forty were 19 to 21 years of age. Five were 22 to 23 years old; one was 26 years of age. Their general health was good and their physical examinations at the beginning of their training showed no defects. As far as was known they came from families that were comfortably off financially.

Initial examination of conjunctiva

The bulbar conjunctiva, which is relatively transparent, contains numerous small blood vessels. It is attached by means of lax connective tissue to the opaque, firm sclera (Fuchs, '24). That it is freely movable on the sclera can be demonstrated by having the patient blink gently while observing the conjunctiva through a slit lamp. Another method of showing the mobility of the conjunctiva is to pull down the lower lid. During either of these procedures the blood vessels in the conjunctiva can be seen to move. The deep conjunctival blood vessels which are presumably attached to the outer surface of the sclera do not move (Wolff, '40). Using the slit lamp, Kruse ('41) described changes in both the superficial and deep parts of the conjunctiva, which he described as transparent, translucent or opaque according to their ability to transmit light. The present authors were unable to see any difference in transparency between the deep and the superficial parts of the conjunctiva. Consequently it was not possible to follow Kruse's method exactly. Instead, the transparency of the conjunctiva was estimated by the clearness with which the deeper, non-movable blood vessels could be seen through a slit lamp. The method used in this study was planned to demonstrate the conjunctival changes described by Kruse.

When examined with the slit lamp, all of the subjects showed some thickening of their bulbar conjunctivae. If one accepts Kruse's ('41) hypothesis, this thickening has occurred because these young women have suffered from a deficiency of vitamin A sometime prior to the examination. In other words,

100% of these subjects showed signs of avitaminosis A, according to Kruse.

As a result of this first examination with the slit lamp, the subjects were divided into three groups depending on the transparency of their bulbar conjunctivae. Group 1 had fairly clear conjunctivae. There were fifteen subjects in this group and five of them showed elevations or pingueculae in one or more of the limbic areas, at the equator (fig. 1). These elevations are raised areas roughly circular or triangular in outline. The elevations, according to Fuchs ('24) are due chiefly to an increase in the number and size of the elastic fibers. In some cases they are of a yellow color, due to the presence of a yellowish hyaline substance. These elevations are thought by ophthalmologists (Parsons, '38) to be more common in individuals exposed to much dust, wind, or other such conditions (see below).

Group 2 had somewhat more opaque conjunctivae and of the thirteen subjects in this group, eight showed elevations.

Group 3 had still more opaque conjunctivae and ten of the twelve subjects in this group showed elevations. Three of these individuals had three to four elevations each, which was more than any of the subjects in groups 1 or 2 showed.

None of the subjects showed markedly opaque conjunctivae or deeply pigmented elevations. Thirty-nine per cent of Kruse's patients showed one or more elevations that were visible to the naked eye, while in the present series, 57% showed such elevations. Kruse's subjects varied in age from 17 to 65 years, and were from low income groups, while in this series they were from 19 to 26 years old and were from higher income groups.

The relation of outdoor sports to the presence of elevations

To check roughly whether there was any relation between indulgence in outdoor sports and the presence of elevations, the subject was asked whether she had gone in for outdoor

sports freely or whether she had taken little of such exercise before coming into training. Then it was determined whether elevations were commoner in the girls who had taken much outdoor exercise and conversely whether the girls who did not indulge in much outdoor exercise rarely had conjunctival elevations. This seemed to be true in general — as 70% of the girls who took much outdoor exercise showed elevations, whereas of the girls who did not indulge in such exercise to any extent only 20% showed elevations. These figures suggest that exposure to wind and the other elements favors the development of these elevations.

Amount of vitamin A in the meals

The nurses received palatable meals which were planned by a dietitian, who held a university degree in Home Economics. As far as could be ascertained no reliable method for determining the total amount of both vitamin A and carotene in the meals was available, but the carotene alone could be fairly readily assayed by a method suggested by Jackson ('43). Carotene determinations were therefore made on 3 successive days each month for the last 9 months of the study. As was expected, the results of the carotene assays showed considerable variation. On 9 of the days the amount of carotene varied from 492 to 1000 μg; on 9 other days it ranged from 1000 to 5000 μg; on 10 days it was between 5000 and 25,000 μg. When these figures are expressed in terms of vitamin A, using the conversion figure of 0.6 μg carotene as equivalent to 1 I.U. of vitamin A, they are as follows: 820 I.U. to 1666 I.U.; 1666 I.U. to 8330 I.U.; 8330 I.U. to 41,650 I.U. The vitamin A Sub-Committee of the Accessory Food Factors Committee (Lister Institute and Medical Research Council) have recently (1945) reported tests on human beings in which only 25 to 40% of the carotene ingested in the form of green or yellow vegetables was retained in the body. It is probable therefore that about one-third of the carotene that is ingested is available to the body.

As we were not able to measure the vitamin A in the food by chemical methods, we estimated, using recent food tables, the amount of it present in the milk, cream, butter, cheese, eggs and liver eaten by a nurse on the same days. The amount of vitamin A in these foods varied from 2500 to 6000 I.U. per day, with an average of 4000 I.U., except on 3 days when it ranged from 12,000 to 14,000 I.U., due to the fact that liver had been served. These figures are approximations, at best, as even the figures given in the food tables show great variations, but they indicate that the meals contained good amounts of vitamin A. Therefore if the conjunctival changes are due to a deficiency of vitamin A, this must have occurred some time previously.

Dosage of vitamin A

Half of each group was given 50,000 I.U. of vitamin A [2] daily in two capsules. Usually half of this was taken at breakfast and the remainder at bedtime. Occasionally the two capsules were taken together at bedtime. The other half of each group received identical looking capsules containing corn oil.[2] Care was taken to see that in each group, half, as nearly as possible, of the subjects with elevations received therapy. All of the nurses were under the impression that they were receiving vitamin A. The authors, who carried out all the examinations, did not know which subjects were receiving vitamin A and which were not. A responsible assistant distributed the capsules every 2 months. The nurses were reminded frequently — that is at least once every few weeks — of the importance of taking the capsules regularly. They also kept records of the number of capsules missed. In regard to the latter, during their annual 3 weeks' holiday and during the 12% of their time that they spent on night duty, the capsules were taken irregularly. During the rest of the year the nurses took them quite faithfully. About 40% reported that they took them very regularly; about 50% missed about

[2] We are indebted to Mead Johnson and Company, for supplies of this material in the form of gelatin capsules.

two per week; and about 10% forgot from three to six capsules per week. However even though some of the nurses forgot to take a fair number of the capsules, the total amount of vitamin A ingested during the 2 years of the test was very great.

Vitamin A blood levels

Blood vitamin A values were determined in thirty of the subjects using the method of May, Blackfan, McCreary and Allen ('40). The first blood samples were taken after the test had been in progress for 9 to 10 months and the blood was drawn in the morning, 2 to 4 hours after the nurse had taken her capsule. The average level in the therapy group was about 9 blue units higher than in the control group (table 1, column 3) — no doubt due to the 25,000 I.U. of vitamin A which had been taken a few hours previously.

TABLE 1

Blood vitamin A values in blue units.

SUBJECTS	NUMBER OF SUBJECTS	AFTER 9–10 MOS. THERAPY		AFTER 22–23 MOS. THERAPY	
		Average	Usual range	Average	Usual range
Taking vitamin A	14	26.8 [1]	21 –33 [1]	22.3 [2]	14–30 [2]
Controls	16	17.9 [1]	12.4–21.5 [1]	21.4 [2]	14–25 [2]

[1] Blood was drawn 2 to 4 hours after 25,000 I.U. of vitamin A had been ingested.
[2] Blood was drawn 11 to 13 hours after 25,000 I.U. of vitamin A had been ingested.

In the second test, after 22 to 23 months on therapy, the nurses were asked to omit the capsule on the morning on which they were to be bled. Consequently the blood was drawn 11 to 13 hours after the last dose of vitamin A had been taken. As is shown in column 5 of table 1, the average blood vitamin A levels of the subjects on therapy and of the controls were very similar, as were also the ranges of the readings. It appears therefore that the addition of 50,000 I.U. of vita-

min A to the daily diet for a period of 22 to 23 months does not raise the blood vitamin A level except for the first few hours after a dose has been taken.

Technique of slit lamp examination

Except during the months of July and August, each nurse was very carefully examined with the slit lamp approximately every 2 months, always by the same observer. Each examination required about 45 minutes' time for each nurse. The subject who was seated in front of the window, but not in direct sunlight, was first examined with the naked eye. The evenness of the surface of the conjunctiva, the number, position, shape and color of the elevations, any enlargement or engorgement of the plica and the extent of the vascular network were recorded. No regular changes in any of these tissues could be observed. This result is contrary to that of Kruse ('41) who described such changes following the ingestion of large amounts of vitamin A daily.

A large scale drawing (8″ x 6″) was made of all the deep bulbar conjunctival vessels that could be seen through the slit-lamp (fig. 1). The fact that the deep vessels do not move when the subject blinks or the observer pulls down the lower lid quickly, allows one to differentiate between the deep and the superficial vessels. The upper lid was held up and the lower lid held down by the subject so that all of the bulbar conjunctiva could be examined. The clarity with which the deep vessels could be seen and the number of them that were visible were used as a measure of the transparency of the conjunctiva. The clearness with which the various parts of the vessels could be seen was indicated by means of numbers written beside the vessels. The clearest parts were designated as 1; those parts just visible as 4, with gradations between designated 2 and 3. After each examination the drawing was traced on a thin sheet of paper — the vessels being shown in red (solid lines in fig. 1) and no figures being copied. At the next examination the vessels seen through the slit lamp were

compared with those shown in the drawing and the clearness with which they were now seen was marked in again by figures. Any new vessels seen at this examination were drawn in with a blue pencil (shown by dotted lines in fig. 1). Any vessels previously recorded and not seen on this examination were surrounded by blue circles and these were omitted in the next tracing. The aim of these drawings was to demonstrate whether the conjunctiva was becoming more transparent and by drawing in the vessels, one could be sure of their loca-

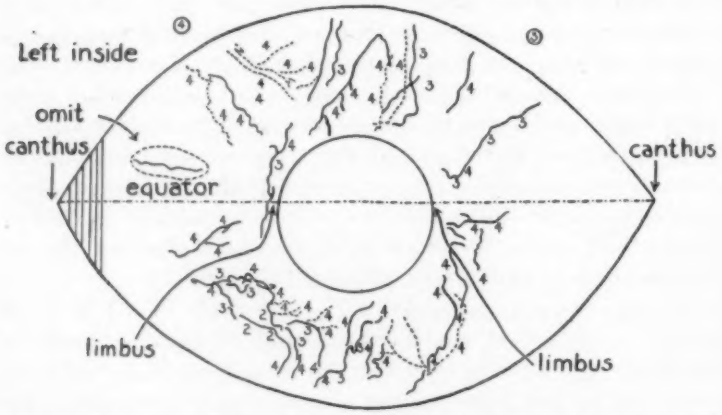

Fig. 1 Large scale drawing of deep conjunctival vessels; the examiner could not see the blood vessels enclosed by the dotted circle at this particular examination, although these vessels were seen in this area in the previous examination. Therefore in the tracing that was made from this drawing these blood vessels were omitted. This tracing served as a guide at the next examination.

tion in the conjunctiva. The subject whose deep conjunctival vessels are shown in figure 1, belonged to the group which had the most opaque conjunctiva (group 3), and therefore few vessels were seen and only parts of two of the vessels were relatively clear (marked 2). No vessels were very clear.

Kruse ('41) states that the thickness of the conjunctiva can often be estimated by counting the number of vessels seen crossing over each other in each zone (area between limbus and canthus). This was therefore done and the number of

vessels seen crossing over each other was recorded above each canthus. These numbers are enclosed in circles in figure 1. For example, 4 means that four strata of vessels crossing over each other could be seen on the average in that zone. This was recorded in the successive examinations but no regular change was noted.

Results of slit lamp examinations after beginning of therapy

At the end of each examination, the record and the drawings of the subject's eyes were compared with her previous drawings and records and the observer decided from this evidence whether more or fewer deep vessels were seen, whether some of these vessels were seen more or less clearly, or whether there was no change.

If the deep vessels were seen more clearly or if more of them were visible, either of which findings would indicate that the conjunctiva was more transparent, the subject was said to be receiving vitamin A. After the whole group of subjects had been examined each time, the assistant, who knew which nurses were receiving vitamin A, checked the results, removed the nurses' names from the sheet and reported in what percentage of the subjects the diagnosis of therapy was correct. From her report it was evident that it was quite impossible to determine by the use of this method who was receiving vitamin A and who was not.

The results of all the slit lamp examinations on each subject were summarized to decide whether any change had occurred from the beginning. Six of the subjects could be kept in the test for only 1 year. Five were on therapy and in three of these the conjunctivae seemed clearer after the year of therapy. The sixth, who was a control, showed no change in her conjunctivae.

Thirty-four of the patients took therapy for 2 years and a summary of the slit-lamp examinations on them is shown in table 2. In the subjects of group 1 (clearest conjunctivae)

none of those on therapy showed clearing, whereas 43% of the controls apparently had clearer conjunctivae at the end than at the beginning of the test. Of the subjects in group 2 (moderately opaque conjunctivae) 40% of those on therapy showed some clearing, but 33% of the controls showed similar clearing. Of the ten subjects with the most opaque conjunctivae (group 3), none showed any clearing, whether they had taken vitamin A or not.

TABLE 2

Percentage of subjects showing more transparent conjunctiva at end of test — determined from 8–9 slit lamp examinations on each subject.

INITIAL APPEARANCE OF CONJUNCTIVA	SUBJECTS GIVEN VITAMIN A (2 YRS.)		CONTROLS	
	Number of subjects	% clearer	Number of subjects	% clearer
Group 1 — Clearest	6	0	7	43
Group 2 — Moderately opaque	5	40	6	33
Group 3 — Most opaque	5	0	5	0

Two of the control subjects showed no change consistently throughout the 2 years of the study. One of the subjects taking vitamin A showed progressive and regular clearing of her conjunctivae but her elevations did not disappear. In all the other thirty-one subjects who were studied for the full 2 years (fifteen on therapy and sixteen controls) the conjunctiva on some examinations seemed to have become more transparent, but on subsequent examinations it seemed to show no change or to have become less transparent.

If the conjunctival thickening is caused by a lack of vitamin A, one would certainly expect that the administration for 2 years of enormous daily doses (50,000 I.U.) of vitamin A would cause the conjunctivae to become definitely thinner. The conjunctivae did not become clearer in these subjects and one would therefore conclude that the thickening was not due to a deficiency of vitamin A.

Photographic studies of conjunctiva

A kodachrome photograph, approximately life size, was taken of each subject's eyes every 6 months. All the conditions, i.e., camera, type of film, flash bulbs, etc., were identical as far as could be determined on each occasion. However the photographs taken at different times varied greatly in their color tones, due to uncontrollable variations in the films and their processing. It was therefore very difficult to compare the pictures taken at different times. Nevertheless at the end of the test after a study of the complete series of pictures of each of the subjects both in a viewing box and by projection in pairs on a smooth screen, a tentative conclusion was reached as to any change in the transparency of the conjunctiva. It

TABLE 3

Percentage of subjects showing more transparent conjunctiva at end of test — as shown by photographs.

INITIAL APPEARANCE OF CONJUNCTIVA	SUBJECTS GIVEN VITAMIN A	CONTROLS
	%	%
Group 1 — Clearest	33	43
Group 2 — Moderately opaque	60	66
Group 3 — Most opaque	40	60

is felt, however, that the photographic records are of doubtful value because of the technical difficulties involved. The results are shown in table 3. No difference could be detected between the nurses on therapy and the controls.

Time lost due to infections

While this study was in progress a record was kept of the number of days the nurses were off work due to illness during the 2 years (table 4). Seven of the sixteen subjects on vitamin A therapy, or 44%, lost time from work because of respiratory infections during the 2 years of the test. These nurses suffered from a total of fifteen infections, which resulted in a loss of 109 working days.

TABLE 4

Incidence of respiratory infections in subjects that did or did not take extra vitamin A.

WITH VITAMIN A		WITHOUT VITAMIN A	
Total no. of nurses given vitamin A	16	Total no. of nurses serving as controls	18
No. of these nurses with respiratory infections	7	No. of these nurses with respiratory infections	11
Per cent of these nurses with respiratory infections	44	Per cent of these nurses with respiratory infections	61
Time lost because of respiratory infections — days	109	Time lost because of respiratory infections — days	198
Number of infections	15	Number of infections	24

DETAILS OF RESPIRATORY INFECTIONS IN NURSES GIVEN VITAMIN A

Name	No. infections	Time off	Diagnosis
		days	
Au	1	5	"Flu"
Ec	2	12	Sore throat, cough
		1	Cold
Jk	3	2	Cold
		2	Cold
		6	"Flu"
N	1	8	Cold
R	5	13	Strept. throat
		9	Cold
		1½	Swollen cerv. glands
		10½	Tonsillitis
		13	Strept. throat
T	1	5	Cold
Ew	2	11	Bronchitis
		10	Cold (fever)
7 pts.	15 infec.	109 days	

DETAILS OF RESPIRATORY INFECTIONS IN CONTROL NURSES

Name	No. infections	Time off	Diagnosis
		days	
Ba	2	30	"Flu" (fever 2 wks.)
		7	"Flu"
Br	3	14	Bronchitis
		4	Cold
		13	Sinusitis
Ed	1	3	Tonsillitis
G	2	5	Cold
		2	"Flu"
Ho	2	1	Cold
		1	Cold
Bk	1	7	Cold
M	4	2	Cold
		7	"Flu"
		14	"Flu"
		15	Cold
Ro	1	14	"Flu"
Ry	1	6	Cold
Sch	4	8	"Flu"
		9	Tonsillitis, sinusitis
		18	Cold
		4	Cold
Tu	3	6	Cold
		3	Laryngitis
		5	Cold
11 pts.	24 infec.	198 days	

The records of the control group (no vitamin A given) were less good. Eleven of these 18 nurses, or 61%, were off duty because of respiratory infections. They suffered from twenty-four respiratory infections, necessitating 198 days off duty.

No record was kept of the slight respiratory infections which did not put the nurses off work.

As for non-respiratory infections, the difference between the two groups was slight. Four nurses in the group receiving therapy lost 50 days' time; seven control nurses lost 59 days' time. These infections included appendicitis, conjunctivitis, food poisoning, "digestive upsets," mumps and infected fingers.

The group receiving therapy did show a lower incidence of incapacitating respiratory infections but the numbers are so small that the results are not conclusive.

CONCLUSIONS

1. The eyes of forty healthy young pupil nurses were examined with a slit-lamp; 100% of them showed thickening of the bulbar conjunctivae. In other words, if one accepts the hypothesis of Kruse ('41), all of these nurses had suffered from avitaminosis A at some time prior to this examination. The forty nurses were then divided into two groups as nearly alike as possible as far as the transparency of their conjunctiva was concerned. One group was given 50,000 I.U. of vitamin A per day in two divided doses. The other group received similar placebos containing corn oil. All of the nurses believed that they were receiving vitamin A and the observer did not know to which group any subject belonged. Thirty-four of the subjects took the capsules for a period of 2 years. Their conjunctivae were examined with a slit lamp at frequent intervals. With the use of this method it was not possible to determine which nurses were receiving vitamin A and which were not, except in the case of three individuals (two controls and one on therapy). In none of the subjects with conjunctival elevations at the beginning did therapy cause the elevations to

disappear. Therefore one would conclude that the conjunctival thickening is not caused by a deficiency of vitamin A.

2. Except for the first few hours after the 25,000 I.U. dose of vitamin A was taken, the blood vitamin A levels were similar to those in the control group.

LITERATURE CITED

FUCHS, E. 1924 Textbook of Ophthalmology. Lippincott, Philadelphia.

JACKSON, S. H. 1943 Personal communication.

KRUSE, H. D. 1941 Medical evaluation of nutritional status. IV. The ocular manifestations of avitaminosis A. Milbank Memorial Fund Quart., vol. 19, p. 207.

MAY, C. D., K. D. BLACKFAN, J. F. MCCREARY, AND F. H. ALLEN, JR. 1940 Clinical studies of vitamin A in infants and in children. Am. J. Dis. Child., vol. 59, p. 1167.

PARSONS, J. H. 1938 Diseases of the Eye. Churchill, London.

Vitamin A Sub-Committee of the Accessory Food Factors Committee (Lister Institute and Medical Research Council) 1945 Nature, vol. 156, p. 11.

WOLFF, E. 1940 The Anatomy of the Eye and Orbit. Blakiston, Philadelphia.

THE NUTRITIONAL SIGNIFICANCE OF ANIMAL PROTEIN SUPPLEMENTS IN THE DIET OF THE CHICK

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It has long been believed that a good poultry diet could not be formulated without including a certain amount of animal protein feeds. That such animal products in the poultry diet are of distinct value is not doubted, but for some time, it has been a thought of ours that perhaps the contribution of these materials is not due so preponderantly to amino acids as has been supposed. More recently it has been established that, in addition to amino acids, minerals, and the better-known vitamins, certain of these animal protein supplements contribute nutritional elements to a practical chick diet, the nature of which is not understood. The indications are that these nutritional effects are due either to still unidentified substances or factors, vitamin-like in character, or to certain little-understood relationships or interactions involving known nutrient materials.

These findings have been an outgrowth of attempts to substitute the more readily available plant protein sources, such as soybeans, for the animal protein feeds which were scarce during the war years. Such attempts led first of all to a recognition of methionine deficiency in soybeans. It is now

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well known that methionine supplements a chick diet which contains soybean oil meal as the major source of protein (Hayward and Hafner, '41; Marvel, Carrick, Roberts and Hauge, '44). Almquist, Mecchi, Kratzer and Grau ('42) stated that heated soybean is slightly deficient in methionine for the chick at the 20% protein level, but is complete in respect to all other amino acids required by the chick.

The value of fish meal in supplementing such a diet is also well known. Christiansen, Deobald, Halpin and Hart ('40) reported that chick rations in which soybean oil meal furnished the majority of the protein produced exceptionally good growth when 3 or 4% fish meal was added. They reported that sardine meal appeared to be superior to menhaden or whitefish meal in this respect. Carver and Evans ('43) stated that a soybean chick starter diet was effectively supplemented by herring fish meal but not by meat scraps. Hammond and Titus ('44) reported that sardine fish meal is of outstanding value as a protein supplement to soybean meal, being superior to meat scrap or dried skim milk. They found that the protein of yeast failed to supplement the protein of soybean. Bird and Mattingly ('45) in tests designed to determine if methionine would supplement their diet as effectively as fish meal, found a significant improvement in growth of chicks when 0.2% dl-methionine was added to a starting and growing mash based largely on corn and soybean oil meal, and reported that the growth stimulus due to the methionine slightly exceeded that obtained by supplementation with 4% fish meal. They raised the question of the possible existence in fish meal of a substance or substances other than methionine, capable of performing, at least in part, the same biological functions as methionine. They did not, however, report feeding fish meal and methionine supplements together. Cravens, McGibbon, and Halpin ('45) found the addition of condensed fish press water or ground fish viscera to be highly effective in supplementing a diet composed of yellow corn, wheat by-products, meat scraps, soybean oil meal, minerals, fish oil and riboflavin.

During the course of research in this laboratory, we have had occasion to investigate certain phases of this problem. Our results are reported here.

EXPERIMENTAL

In an attempt to demonstrate the existence of an unidentified factor in fish meal, as indicated by work employing practical diets based largely on corn and soybeans, a purified diet was fed ad libitum to single-comb White Leghorn cockerels. These chicks were fed in groups of ten, in electrically-heated metal cages in an air-conditioned room. The percentage composition of the purified ration was as follows: casein, vitamin-free 18.0; gelatin 10.0; soybean oil 5.0; gelatinized starch 52.7; and gelatinized starch plus supplements q.s. to total 100. The supplements, in milligrams per 100 gm of ration, were as follows: NaCl, 836.8; K_2HPO_4, 1611.1; $CaHPO_4 \cdot 2H_2O$, 1374.7; $MgSO_4$, 249.0; $CaCO_3$, 1498.7; $FeC_6H_5O_7 \cdot 6H_2O$, 137.4; $MnSO_4 \cdot 4H_2O$, 40.0; $ZnCl_2$, 1.25; $CuSO_4 \cdot 5H_2O$, 1.5; KI, 4.0; l-cystine, 200.0; thiamine hydrochloride, 0.3; riboflavin, 0.6; pyridoxine hydrochloride, 0.4; dl-calcium pantothenate, 3.0; nicotinic acid, 10.0; 2-methyl-1,4-naphthoquinone, 0.5; i-inositol, 100.0; biotin, 0.015; choline chloride, 150.0. Halibut liver oil containing 60,000 U.S.P. units of vitamin A and 1,000 U.S.P. units of vitamin D per gram, fortified by the addition of 1,500 A.O.A.C. units of vitamin D_3 per gram and 10.5 mg alpha-tocopherol acetate per gram, was fed orally at the rate of 2 drops per week.

The results obtained on this purified ration are shown in table 1. The basal group failed to grow, as was expected, due to a lack of "folic acid." No improvement resulted from the addition of fish meal to the purified ration,[3] contrary to the growth benefits noted with a corn-soybean ration. As a positive control, yeast extract, which has been found to be of little benefit as a supplement to the corn-soybean ration, permitted good growth on the purified diet.

[3] Cravens, McGibbon and Halpin ('45) have since reported that condensed fish press water failed to improve growth on a purified diet.

In another part of our investigations, a semi-practical ration based largely on corn and soybean oil meal was employed, and various supplements were used in our attack upon the problem. The basal rations used are shown in table 2. All of these rations were calculated to contain 18% crude protein. Fat-soluble vitamins were fed orally at one-half the level used with the purified ration.

TABLE 1

Experiments with purified ration.

SUPPLEMENTS	AVERAGE BODY WEIGHT			
	1 week	2 weeks	3 weeks	4 weeks
	gm	*gm*	*gm*	*gm*
First experiment[1]				
None	57.1	72.7	83.6	80.0 (8 dead)
Menhaden fish meal, 3%	54.2	69.5	83.2	84.5 (6 dead)
Second experiment				
None	66.0	87.8	80.0	... (10 dead)
Sardine fish meal, 2%	65.8	93.8	110.5	... (10 dead)
Sardine fish meal, 4%	62.4	85.6	104.3	.. (10 dead)
Yeast extract, 1%	73.6	121.8	185.2	268.8

[1] Experiments were carried out with groups of ten chicks for each ration.

TABLE 2

Basal rations used.

RATION NUMBER	1	2	3	4	5
Ingredients in per cent:					
Ground whole yellow corn	69.5	71.1	72.5	71.1	72.5
Heated extracted soybean oil meal, 44% protein	26.0	22.4	19.0	22.4	19.0
Pacific sardine fish meal, 70% protein		2.0	4.0		
Casein-gelatin-cystine mixture				2.0	4.0
Corn starch plus supplements	2.0	2.0	2.0	2.0	2.0
Tricalcium phosphate	2.0	2.0	2.0	2.0	2.0
Iodized salt	0.5	0.5	0.5	0.5	0.5
Supplements in mg per 100 gm ration:					
Manganese sulphate	20.0	20.0	20.0	20.0	20.0
Riboflavin	0.3	0.3	0.3	0.3	0.3
Pyridoxine hydrochloride	0.3	0.3	0.3	0.3	0.3
2-Methyl-1,4-Naphthoquinone	0.04	0.04	0.04	0.04	0.04

RESULTS

The first step in our experimentation was to determine the extent to which methionine was able to supplement the basal ration (Ration no. 1). In repeated trials with this diet, we have found that the maximum effect of methionine supplementation can be obtained with 0.15%, or at the most 0.30%,

TABLE 3

Effect of methionine on basal ration.

RATION	ADDED METHIONINE	AVERAGE BODY WEIGHT			
		1 week	2 weeks	3 weeks	4 weeks
no.	%	gm	gm	gm	gm
1	0.0	64.4	113.2	178.7	247.1
1	0.15	68.4	121.2	198.8	291.8
1	0.30	68.4	122.6	202.2	301.8
1	0.60	70.2	124.2	200.8	301.4
1	1.20	67.4	111.8	170.8	241.8

TABLE 4

Results with methionine and fish meal.

RATION	ADDED METHIONINE	AVERAGE BODY WEIGHT			
		1 week	2 weeks	3 weeks	4 weeks
no.	%	gm	gm	gm	gm
1		65.2	103.2	154.2	202.0
1	0.15	57.8	116.0	182.0	252.0
1	0.30	71.8	120.7	183.6	252.2
2		71.6	127.0	203.6	304.2
2	0.15	74.2	128.2	207.1	305.1
2	0.30	69.5	125.1	206.4	299.7
3		69.4	127.6	203.2	306.8
3	0.15	75.4	130.2	209.8	305.4
3	0.30	72.4	120.6	191.4	282.8

of dl-methionine; 0.60% was no improvement over 0.30%, and 1.20% methionine caused growth retardation. One such trial is presented in table 3. Consequently, in the subsequent trials with methionine and other supplements methionine has been fed at the 0.15 and 0.30% levels only.

The results of the next experiment, involving fish meal as well as methionine, are shown in table 4. In order to vis-

ualize more clearly the differences between total weight gains at 4 weeks due to methionine and fish meal as independent variables, the growth increment differences were obtained, by subtraction, from the 4 weeks' column of table 4, and are presented in table 5, column 1.

Since these increments show a beneficial effect of fish meal, even in the presence of an optimal level of methionine, the thought occurred to us that this beneficial effect might be due to the presence of known vitamins in the fish meal. In an attempt to rule out this possibility, the ration was supplemented with the following ingredients (milligram per 100

TABLE 5

Growth increments at 4 weeks.

WEIGHT GAIN DUE TO 0.3% METHIONINE IN THE PRESENCE OF VARYING AMOUNTS OF FISH MEAL	Column 1	Column 2
	gm	gm
Ration no. 1 (no fish meal)	+ 50.2	+ 42.6
Ration no. 2 (2.0% fish meal)	− 4.5	− 13.2
Ration no. 3 (4.0% fish meal)	− 24.0	− 1.4

WEIGHT GAIN DUE TO 2.0% FISH MEAL IN THE PRESENCE OF VARYING AMOUNTS OF METHIONINE	Column 1	Column 2
	gm	gm
No methionine added	+ 102.2	+ 87.8
0.15% methionine	+ 53.1	+ 38.0
0.30% methionine	+ 47.5	+ 32.0

gm of ration): thiamine hydrochloride, 0.3; riboflavin, 0.6; pyridoxine hydrochloride, 0.4; dl-calcium pantothenate, 3.0; nicotinic acid, 10.0; 2-methyl-1,4-naphthoquinone, 0.5; i-inositol, 100.0; p-aminobenzoic acid, 3.0; biotin, 0.015; and choline chloride, 150.0. When this supplement was used, the riboflavin, pyridoxine and 2-methyl-1,4-naphthoquinone levels listed in the basal ration were omitted. Vitamins A, D_3, and alpha-tocopherol were supplied orally as usual.

The results in the presence of this supplement were consistent with the previous results, as shown in table 5, column 2. This indicated that the effect of fish meal in all probability

was not due to any of the constituents included in the supplement, or methionine.

Our next step was to add 0.5% yeast extract[4] to the diet in addition to the supplement listed above. This was done in order to provide a source of folic acid activity, and also because Hill, Scott, Norris and Heuser ('44) had reported the possibility of a plant protein diet being deficient in unidentified factors supporting chick growth which could be supplied by feeding dried brewers' yeast. Our results with yeast extract are shown in table 6.

TABLE 6

Effect of yeast extract.

RATION no.	SUPPLEMENTS	AVERAGE BODY WEIGHT			
		1 week	2 weeks	3 weeks	4 weeks
		gm	*gm*	*gm*	*gm*
1	Vitamins, choline	71.4	120.4	178.8	250.8
1	Vitamins, choline, 0.30% methionine	74.4	128.4	201.8	293.4
1	Vitamins, choline, 0.5% yeast extract	76.4	127.8	204.0	286.0
1	Vitamins, choline, 0.30% methionine, 0.5% yeast extract	72.4	125.0	198.8	289.2

We next attempted to determine the extent to which the amino acids present in the fish meal protein were responsible for its growth stimulation. Using the amino acid analysis from Block and Bolling ('45), by calculation it was determined that a mixture containing 1296 gm of vitamin-free casein, 144 gm of gelatin and 5 gm of cystine should provide the amino acids known to be required by chicks at levels approximately equal to those contained in fish meal. Such a mixture was prepared, and substituted for fish meal at equivalent levels in rations 4 and 5 (table 7).

[4] Standard Brands Type III.

TABLE 7

Effect of casein mixture.

RATION		AVERAGE BODY WEIGHT			
		1 week	2 weeks	3 weeks	4 weeks
no.		gm	gm	gm	gm
1	(basal)	63.4	106.4	153.0	197.8
2	(2.0% fish meal)	66.6	118.8	202.8	294.6
3	(4.0% fish meal)	68.6	124.8	202.8	306.9
4	(2.0% casein mixture)	60.6	98.2	147.4	211.2
5	(4.0% casein mixture)	63.6	103.6	159.4	212.6

DISCUSSION

From the data in tables 4 and 5 it appears evident that sardine fish meal supplements the basal diet more completely than methionine. In the presence of fish meal, added methionine was of little value, although methionine did supplement the diet, to a lesser extent, when fish meal was absent. On the other hand, a diet containing optimal levels of methionine, according to our findings, was still incomplete and benefitted materially from further supplementation with fish meal. This beneficial effect of fish meal occurred over and above any supplementary effect of methionine, choline, or any of the identified vitamins known to be required for chick growth.

From table 6 it appears that the addition of 0.5% yeast extract to the basal diet containing the vitamin mixture plus choline did exert some positive effect upon growth. However, a 0.30% methionine supplement likewise increased growth without yeast extract, and the presence of yeast extract in addition to methionine did not induce greater growth than that obtained with methionine alone. Hence we have concluded that the unknown factor present in sardine fish meal which supplemented the rations containing 0.30% methionine is probably not directly related to the unknown factor in brewers' yeast.

The casein-gelatin-cystine mixture (table 7) failed to increase gain in weight significantly above the basal level, whereas the fish meal levels fed as positive control groups repeated past performance. From this evidence it appears

doubtful if a deficiency of amino acids other than methionine is likely to be the first limiting factor in our basal ration. As further evidence, we have obtained, in preliminary work, a water-soluble fraction from sardine fish meal which effectively supplements the basal ration when added at low levels, as shown in table 8.[5] We have attempted to eliminate the probability that choline, methionine, known vitamins, or unknown factors present in yeast extract, are responsible to any major extent for the effect produced with sardine fish meal. Our trials with the casein mixture, and with the fish meal fraction, tend to indicate that we are dealing with an unknown factor,

TABLE 8

Effect of fish meal fraction.

RATION	FISH MEAL FRACTION	AVERAGE BODY WEIGHT			
		1 week	2 weeks	3 weeks	4 weeks
no.	%	*gm*	*gm*	*gm*	*gm*
1	...	68.2	105.6	166.8	220.2
1	0.06	75.8	122.0	211.6	298.8
1	0.12	72.2	115.6	201.6	300.8
2	...	73.2	124.6	212.0	332.2
3	...	74.4	128.6	222.0	324.6

present in sardine fish meal, and required at the low levels commonly associated with vitamins rather than amino acids.

The results with the purified ration, however, are difficult to reconcile since it appears that the postulated unknown factor in fish meal is needed for growth on the corn-soybean basal diet but is of no value when added to a purified diet. It might be presumed that this failure was due to a primary lack of "folic acid" in the purified diet, which was not supplied by the fish meal, and because of this the secondary deficiency of another unknown factor did not manifest itself. This is hardly likely in view of the results with yeast extract, since it would be necessary to assume then that yeast extract

[5] Berry, Carrick, Roberts and Hauge ('45) have since reported that a water extract of fish press water containing only a small percentage of protein had most of the supplementary value of whole fish press water.

contained the same unknown factor (in addition to "folic acid") as fish meal and this did not appear to be the case from the feeding trials in which yeast extract was added to the corn-soybean ration. Another alternative is that the casein or gelatin could be a source of the unknown factor in the purified diet, but this hypothesis fails in view of the negative results obtained when casein and gelatin were used to replace fish meal in the corn-soybean diet.

A more likely explanation is that fish meal increases chick growth on a corn-soybean diet by supplying some factor, known or unknown, whose requirement has been augmented, or created, by the presence of corn and/or soybean oil meal in the diet. This would explain the failure to discover any such factor using the purified diet. If this should prove to be the case, the factor present in sardine fish meal would be of great practical importance nevertheless, since corn and soybeans are likely to continue to enjoy widespread use in poultry diets.

SUMMARY

A study was made of the extent to which dl-methionine and sardine fish meal are able to supplement a chick ration containing proteins from corn and soybeans only. Such a diet appears to be lacking in an unknown growth factor which is present in sardine fish meal. There is some evidence that the need for this factor is a peculiarity of the corn-soybean diet.

LITERATURE CITED

ALMQUIST, H. J., E. MECCHI, F. H. KRATZER AND C. R. GRAU 1942 Soybean protein as a source of amino acids for the chick. J. Nutrition, vol. 24, p. 385.

BERRY, E. P., C. W. CARRICK, R. E. ROBERTS AND S. M. HAUGE 1945 Condensed fish press water and fish liver meal in chick rations. Poultry Sci., vol. 24, p. 270.

BIRD, H. R., AND J. P. MATTINGLY 1945 Addition of dl-methionine to starting and growing mashes. Poultry Sci., vol. 24, p. 29.

BLOCK, R. J., AND DIANA BOLLING 1945 The Amino Acid Composition of Proteins and Foods. Charles C. Thomas, Springfield, Illinois.

CARVER, J. S., AND R. J. EVANS 1943 Animal and vegetable protein combinations in chick starting ration. The U. S. Egg and Poul. Mag., vol. 49, p. 468.

CHRISTIANSEN, J. B., H. J. DEOBALD, J. G. HALPIN AND E. B. HART 1940 Practical supplements for soybean oil meal in chick rations. Poultry Sci., vol. 19, p. 18.

CRAVENS, W. W., W. H. MCGIBBON AND J. G. HALPIN 1945 The value of certain supplements in practical chick rations containing adequate riboflavin. Poultry Sci., vol. 24, p. 305.

HAMMOND, J. C., AND H. W. TITUS 1944 The use of soybean meal in the diet of growing chicks. Poultry Sci., vol. 23, p. 49.

HAYWARD, J. W., AND F. H. HAFNER 1941 The supplementary effect of cystine and methionine upon the protein of raw and cooked soybeans as determined with chicks and rats. Poultry Sci., vol. 20, p. 139.

HILL, F. W., M. L. SCOTT, L. C. NORRIS AND G. F. HEUSER 1944 Deficiency of unidentified vitamins in practical chick rations. Poultry Sci., vol. 23, p. 253.

MARVEL, J. A., C. W. CARRICK, R. E. ROBERTS AND S. M. HAUGE 1944 The supplementary value of choline and methionine in a corn and soybean oil meal chick ration. Poultry Sci., vol. 23, p. 294.

PANTOTHENIC ACID DEFICIENCY AND REPRODUCTION IN THE RAT[1]

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The importance of pantothenic acid for normal reproduction and embryonic development in the chick has been stressed by several investigators, e.g., Bauernfeind and Norris ('39), Gillis, Heuser, and Norris ('42), and Pearson et al. ('45). However, little is known concerning the effects of pantothenic acid deficiency on reproduction in rats. In general, it may be said that in chronic nutritional deficiencies with prolonged survival, the oestrous cycle is irregular or absent (Evans and Bishop, '22 a). In severe, acute deficiency in pantothenic acid it is known that the majority of animals do not mature before they die (Figge and Allen, '42). The study of reproductive phenomena in partial prolonged pantothenic acid deficiency has not, as far as we are aware, been reported by any investigator.[2]

If a pantothenic acid deficient diet is given to rats on the day of parturition, little interference with lactation is shown since 83% of the young are weaned.[3] Therefore, it seemed

[1] Aided by grants from the Board of Research and from the Department of Agriculture of the University of California, and the Rockefeller Foundation, New York City. The following materials were generously contributed: crystalline B vitamins from Merck and Company, Inc., Rahway, New Jersey; synthetic alpha-tocopherol from Hoffmann-LaRoche Company, Nutley, New Jersey.

[2] In this laboratory we have occasionally succeeded in breeding female rats deficient in the filtrate fraction of the B complex but failure of implantation or resorptions has always resulted (Evans and Emerson, unpublished).

[3] Nelson, M. M., and H. M. Evans — In preparation.

of interest to place normal female rats on pantothenic acid deficient diets at intervals during gestation or even before mating to see whether any upsets in the reproductive function would occur. Jukes ('40) mentioned the occurrence of resorptions in part of a group of stock rats placed on pantothenic acid deficient diets at mating. Sure ('41) noted that reproduction on purified diets without the addition of calcium pantothenate was very abnormal since either "sterility" of the mother or a high incidence of still births in the young resulted.

EXPERIMENTAL

Normal female rats, 2 to 5 months of age, were bred with normal males and placed on the experimental diets the first or the thirteenth day of gestation. Other groups of rats were maintained on pantothenic acid deficient diets for 15 days and then bred as rapidly as possible. Paired-feeding studies were carried out with some groups. Vaginal smears were examined during gestation for the presence of erythrocytes, the sign that implantation has occurred; all rats were weighed at regular intervals. The deficient and control groups were carefully balanced according to body weight and age at the time of breeding. All rats were kept in cages with screens until the day before littering was expected to occur.

Two basal deficient diets, differing only with respect to the levels of the B vitamins and to the composition of the salt mixture, were used. Both diets were composed of 24% alcohol-extracted casein, 64% sucrose, 8% hydrogenated cottonseed oil,[4] and 4% salts. The deficient diet 841 contained McCollum salts No. 185[5] and the following B vitamins per kilogram diet: thiamine HCl 2 mg, pyridoxine HCl 2 mg, riboflavin 5 mg, p-aminobenzoic acid 5 mg, nicotinic acid 10 mg, inositol 200 mg, and choline HCl 0.5 gm. The corresponding control diet 831 contained, in addition, 28 mg calcium pantothenate per kilogram diet. These vitamins were added to the diet in the form of a 20% alcoholic

[4] Crisco.

[5] McCollum and Simmonds ('18).

solution. The deficient diet 846 contained an improved salt mixture, salts No. 4,[6] and higher levels of the same B vitamins; thiamine HCl 5 mg, pyridoxine HCl 5 mg, riboflavin 10 mg, p-aminobenzoic acid 10 mg, nicotinic acid 20 mg, inositol 400 mg, and choline HCl 1.0 gm. The corresponding control diet 836 contained, in addition, 50 mg calcium pantothenate per kilogram diet. These vitamins were added to the diet as a dry mixture with sucrose. Fresh batches of all diets used were made up weekly, placed in dark bottles, and kept in the refrigerator except when the diet was being fed. Every deficient and control rat received weekly a fat-soluble vitamin mixture by stomach tube or in supplement cups that furnished a minimum of 400 U.S.P. units vitamin A, 58 A.O.A.C. Chick Units vitamin D, 3 mg synthetic alpha-tocopherol,[7] and 325 mg corn oil.[8]

Experimental diets started the thirteenth day of gestation

There was no significant difference in reproductive performance between the deficient and control groups as judged by the average number of young born and their average weight at birth (table 1).

Experimental diets started the first day of gestation

Marked upsets in the reproductive function were observed with this procedure. Approximately one-third of the rats in both deficient groups underwent resorptions instead of littering (table 1). This is in agreement with the findings of Jukes ('40). These resorptions were confirmed in each case at autopsy by finding several sites of resorption in the uterus. The remainder of the deficient rats littered but the average weight of the young at birth was significantly decreased. In the case of the rats maintained on the deficient diet 841, the average number of young per litter was also decreased. In

[6] Hegsted et al ('41).
[7] Hoffmann-LaRoche.
[8] Mazola.

TABLE 1

Effect of pantothenic acid deficiency on reproduction.

	EXPERIMENTAL GROUP	DIET NO.	NO. OF RATS BRED	AGE WHEN BRED	FAILED IMPLANTATION	RESORPTIONS OF IMPLANTATIONS	LITTERS OF IMPLANTATION	TOTAL NO. YOUNG	YOUNG BORN DEAD	AV. SIZE OF LITTER	AV. WT. YOUNG
				days	%	%	%		%		*gm*
PANTOTHENIC ACID DEFICIENT	13th day gestation	841	9	92 (69–123)	0	0	100	83	0	9.2(5–12)	5.4
		846	10	99 (70–126)	0	0	100	87	0	8.7(4–11)	5.9
	1st day gestation	841	10	112 (85–159)	0	30	70	48	6	6.9(6– 8)	4.8
		846	11	128(103–155)	0	36	64 [2]	61	10	8.7(4–13)	4.6
	16–22 days before gestation	841	11	129(117–142)	27	50	50 [3]	27	4	6.8(5– 9)	4.3
		846	9	127(123–131)	33	100	0	..	..	..	..
PANTOTHENIC ACID CONTROLS	13th day gestation	831	10	88 (69–123)	0	0	100	89	0	8.9(7–11)	5.6
		836	11	98 (70–126)	0	0	100	104	0	9.5(8–12)	6.3
	1st day gestation	831	11	116 (86–159)	0	0	100	102	1	9.3(4–15)	5.5
		836	10	121 (96–134)	0	0	100	91	11 [4]	9.1(3–15)	5.5
	16–23 [1] days before gestation	841 + CaP	10	132(120–143)	10	0	100	72	6	8.0(3–10)	5.9
		836	10	130(118–143)	0	0	100	83	0	8.3(4–13)	5.7

[1] These rats were maintained on the deficient diet for 16–23 days before breeding; control diets were started the day of breeding.

[2] Two of these seven litters were found at autopsy; they consisted of some living fetuses, some dead and others in the process of resorption.

[3] One of these four litters was found at autopsy; it consisted of some living fetuses, some dead and others in the process of resorption.

[4] These dead young were in one litter only.

the group on the deficient diet 846 two of the seven litters were found in the uterus at autopsy and consisted of some living fetuses, some dead, and others in the process of resorption.

Experimental diets started 16–23 days before breeding

In this experiment all rats were placed on the deficient diets for 15 days. Then they were bred as fast as possible with normal males. Half of them were continued on the deficient diets and the other half placed on the control diets the day of breeding. The average length of time for all rats on the deficient diet before breeding was 18 days for diet 841 and 19 days for diet 846.

Approximately one-third of the deficient rats failed to implant (table 1), thus indicating a marked interference with the reproductive mechanism. Of the rats maintained on diet 841, 50% of the implantations underwent resorption, while 100% of the implantations on diet 846 resorbed. This was confirmed in each case by examination of the uterus at autopsy. Of the four litters that were obtained from rats maintained on diet 841, one of them was found in the uterus at autopsy and consisted of some living fetuses, some dead, and others in the process of resorption. Furthermore, the average number of young per litter and their average weight at birth were significantly decreased in comparison with control values.

In the "control" rats, which received pantothenic acid on the day of breeding, the only indications of damage to the reproductive mechanism from maintenance on the deficient diets for 2 to 3 weeks before breeding were of doubtful significance, i.e., the slightly decreased number of young per litter and the failure of implantation for one rat maintained previous to breeding on diet 841.

Effect of limited dietary intake on gestation

To eliminate the factor of undernutrition, pair-fed controls were added to the previous experimental procedure. Of the

rats maintained on the deficient diet 841 for 15 days before breeding, one-third of them were continued on the deficient diet and their dietary intake measured daily; one-third of them received 1 mg calcium pantothenate daily plus the same quantity of diet 841 consumed by the corresponding deficient rats on the same day of gestation; and the remaining one-third received 1 mg calcium pantothenate daily plus diet 841 ad libitum. The rats were carefully paired in respect to age and body weight on the day of mating and with regard to the weight change during the first 15 days on the deficient diet. To guard against the possibility of a vitamin E deficiency, the quantity of fat-soluble vitamins given weekly was doubled.

The experimental data are shown in table 2. In the deficient group three rats failed to implant, four rats resorbed, and four rats littered. In the control group restricted in calories, only two rats failed to implant and the remainder of the rats cast litters. The restriction in calories varied from 43–84% (average 69%) of the food intake by rats given the diet ad libitum. The increased efficiency of food utilization by rats receiving pantothenic acid can be seen by comparing the weight changes during the 22-day gestation period of the deficient and caloric control rats, regardless of the occurrence of implantation.

The average number of young per litter and their average weight at birth were the same for both control groups, despite the difference in food intake. However, the rats receiving the diet ad libitum gained much more during gestation than did the rats restricted in calories.

Growth and survival of suckling young rats

The growth and survival of the deficient suckling young born under the experimental conditions are shown in table 3. The data for young deficient in pantothenic acid from birth are included for comparison. Increasing the period of the deficiency decreased both growth and survival proportionally. In every case the use of the deficient diet 846 instead of diet 841 seemed to accentuate the deficiency. This may also be

TABLE 2

Effect of pantothenic acid deficiency and limited caloric intake on reproduction deficient diet started 16–23 days before gestation.

EXPERIMENTAL GROUP	RAT	BODY WT. AT MATING	WT. CHANGE DURING GESTATION [1]	AV. DAILY FOOD INTAKE [1]	RESULT OF BREEDING			
					Implantation	No. implantation sites	No. of young cast [2]	Av. wt. young
		gm	gm	gm				gm
Diet 841	1	196	0	9.9	—	0		
pantothenic	2	200	— 22	7.3	—	0		
acid deficient	3	211	+ 7	10.2	—	0		
	4	223	— 5	12.8	+	8	0	
	5	235	— 34	8.6	+	7	0	
	6	244	+ 16	14.3	+	8	0	
	7	268	+ 13	12.9	+	12	0	
	8	240	+ 32	11.9	+	9	4 [3]	4.0 [3]
	9	250	+ 48	13.8	+	10	8 + 1	4.8
	10	265	+ 44	13.3	+	11	5	5.4
	11	296	+ 12	10.4	+	14	9	3.8
Diet 841 + CaP [4]	1	186	+ 4	9.9	—	0		
paired	2	202	— 2	7.4	+	8	3	5.7
feeding	3	201	+ 38	10.2	+	9	9	5.2
controls	4	220	+ 63	12.7	+	11	10	5.2
	5	236	— 19	8.6	—	0		
	6	240	+ 87	14.3	+	11	11	5.6
	7	270	+ 68	13.0	+	9	9	6.0
	8	242	+ 65	11.9	+	10	8	6.1
	9	255	+ 71	13.8	+	9	9	5.3
	10	259	+ 69	13.3	+	10	9	5.6
	11	280	+ 45	10.4	+	8	7	5.7
Diet 841 + CaP [4]	1	175	+ 22	13.2	—	0		
ad libitum	2	202	+ 74	13.3	+	10	9	5.9
controls	4	218	+ 96	17.1	+	10	9	5.9
	5	236	+ 126	20.1	+	7	7	6.1
	6	246	+ 99	17.0	+	11	0 + 3	5.0
	7	258	+ 84	17.8	+	10	10	6.3
	8	244	+ 84	16.6	+	8	5 + 2	5.4
	9	256	+ 96	18.8	+	8	8	5.8
	10	262	+ 100	18.4	+	9	9	5.8
	11	270	+ 99	16.3	+	10	10	6.2

[1] Data determined during the 22 days following mating.

[2] Living plus dead.

[3] These young were living foetuses found in the uterus at autopsy.

[4] 1 mg calcium pantothenate daily.

TABLE 3

Effect on growth and survival of young from mothers on pantothenic acid deficient diets instituted at parturition or during pregnancy.

EXPERIMENTAL GROUP	DIET NO.	NO. OF YOUNG	AV. WT. DAY 1	AV. WT. DAY 10	SURVIVAL DAY 10	AV. WT. DAY 21	SURVIVAL DAY 21	AV. WT. DAY 30	SURVIVAL DAY 30	AV. WT. DAY 60	SURVIVAL DAY 60	AV. LIFE IN DAYS
			gm	gm		gm		gm		gm		
Day of birth	841	175 ♂	6.1	16.6	95%	28	84%	31	61%	47	13%	35.4 ± 1.3[1]
		153 ♀	5.9	16.7		27		29		45		(5–109)
	846	40 ♂	6.3	17.9	99%	30	81%	32	33%	51	1%	28.9 ± 1.3
		38 ♀	6.0	16.6		27		31				(5– 76)
13th day of gestation	841	29 ♂	5.8	14.6	79%	19	23%		0%			15.7 ± 0.9
		24 ♀	5.4	14.3		21						(5– 27)
	846	31 ♂	6.1	17.3	96%		0%					14.5 ± 0.5
		24 ♀	5.7	16.0								(2– 20)
1st day of gestation	841	17 ♂	5.4	11.0	20%		0%					6.1 ± 0.7
		23 ♀	4.7	14.0								(1– 15)
	846	11 ♂	5.7		0%							4.0 ± 0.5
		11 ♀	6.0									(1– 9)

[1] Standard error of the mean.

noted in the data on reproductive performance (table 1). This effect may be due to improvements in the basal diet which improve growth and so increase the pantothenic acid requirement or to the presence in larger amounts of some factor accentuating pantothenic acid deficiency such as nicotinic acid (Morgan and Simms, '39) or choline (Mills et al., '40).

DISCUSSION

Many different dietary conditions have been reported to cause resorption in the pregnant female rat: inanition (Barry, '20), vitamin E deficiency (Evans and Bishop, '22 b), vitamin A deficiency (Sure, '28), a deficiency in the essential fatty acids (Burr and Burr, '30), a low protein intake (Guilbert and Goss, '32), vitamin B deficiency (Ueno, '34), filtrate factor deficiency,[9] pantothenic acid deficiency (Jukes, '40), riboflavin deficiency (Warkany and Nelson, '42), tryptophane deficiency (Albanese, Randall and Holt, '43), and very recently, biotin deficiency (Kennedy and Palmer, '45). In some of the studies reported the interpretation of results is difficult because of the concurrent effects of inanition and of one or more specific dietary deficiencies. This is particularly true in the case of the vitamin B deficiency reported by Ueno ('34). Furthermore, all early work done on inanition probably included qualitative as well as quantitative deficiencies.

In the case of pantothenic acid deficiency, the experimental data given here shows that restriction of calories to 69% (or lower in a few cases) of the normal intake did not produce resorptions. In the deficient groups of rats that resorbed, the average daily food intake averaged 12.2 gm daily or 69% of the calorie intake of controls given the diet ad libitum whereas the deficient rats that littered averaged 12.4 gm daily or 70% of the ad libitum intake, thus revealing a lack of correlation between food intake and reproductive behavior. Furthermore, the possibility of dietary deficiencies other than that of pantothenic acid as a cause of resorptions under these

[9] Evans and Emerson, unpublished.

conditions can be eliminated by the fact that the pantothenic acid-fed group restricted in calories did not undergo resorptions. With regard to the specific question of adequate vitamin E, the deficient rats in the last experiment received a minimum of 18 mg alpha-tocopherol by the fifth day following mating. On the usual unpurified high-fat E-low diet used for bioassays 3 mg of alpha-tocopherol will prevent resorptions in standardized E-low females (Evans et al., '36). On purified low-fat diets the requirement for vitamin E is decreased (Gottlieb et al., '43).

SUMMARY

Reproduction has been studied in normal adult female rats placed on purified diets deficient in pantothenic acid before or during the gestation period.

Pantothenic acid deficiency instituted on the thirteenth day of gestation did not interfere with the reproductive function. Pantothenic acid deficiency instituted 16–23 days before mating or as late as the day of mating always resulted in failure of implantation, resorption, or defective litters.

The normality of reproduction in pantothenic acid-fed controls restricted in calories eliminated the factors of inanition and of other specific dietary deficiencies as causes for these marked upsets in reproduction.

LITERATURE CITED

ALBANESE, A. A., R. M. RANDALL AND L. E. HOLT, JR. 1943 Effect of tryptophane deficiency on reproduction. Science, vol. 97, p. 312.

BARRY, L. W. 1920 The effects of inanition in the pregnant albino rat, with special reference to the changes in the relative weights of the various parts, systems and organs of the offspring. Contrib. Embryology Carnegie Inst., vol. 11, p. 91.

BAUERNFEIND, J. C., AND L. C. NORRIS 1939 The antidermatosis vitamin required for reproduction in the domestic fowl. Science, vol. 89, p. 416.

BURR, G. O., AND M. M. BURR 1930 On the nature and role of the fatty acids essential in nutrition. J. Biol. Chem., vol. 86, p. 587.

EVANS, H. M., AND K. S. BISHOP 1922 a On the relations between fertility and nutrition. II. The ovulation rhythm in the rat on inadequate nutritional regimes. J. Metabolic Research, vol. 1, p. 335.

———— 1922 b On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science, vol. 56, p. 650.

EVANS, H. M., O. H. EMERSON AND G. A. EMERSON 1936 The isolation from wheat germ oil of an alcohol, alpha-tocopherol, having the properties of vitamin E. J. Biol. Chem., vol. 113, p. 319.

FIGGE, F. H. J., AND E. ALLEN 1942 Genital atrophy during pantothenic acid deficiency and responses to gonadotropic and estrogenic hormones. Endocrinology, vol. 30, p. S 1028.

GILLIS, M. B., G. F. HEUSER AND L. C. NORRIS 1942 The need for pantothenic acid and an unidentified factor in reproduction in the domestic fowl. J. Nutrition, vol. 23, p. 153.

GOTTLIEB, H., F. W. QUACKENBUSH AND H. STEENBOCK 1943 The biological determination of vitamin E. J. Nutrition, vol. 25, p. 433.

GUILBERT, H. R., AND H. GOSS 1932 Some effects of restricted protein intake on the estrus cycle and gestation in the rat. J. Nutrition, vol. 5, p. 251.

HEGSTED, D. M., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1941 Choline in the nutrition of chicks. J. Biol. Chem., vol. 138, p. 460.

JUKES, T. H. 1940 Reproduction in rats on synthetic B-complex supplement. Proc. Soc. Exp. Biol. Med., vol. 45, p. 625.

KENNEDY, C., AND I. S. PALMER 1945 Biotin deficiency in relation to reproduction and lactation. Archiv. Biochem., vol. 7, p. 9.

MCCOLLUM, E. V., AND N. SIMMONDS 1918 A study of the dietary essential, water-soluble B, in relation to its solubility and stability toward reagents. J. Biol. Chem., vol. 33, p. 63.

MILLS, R. C., J. H. SHAW, C. A. ELVEHJEM AND P. H. PHILLIPS 1940 Curative effect of pantothenic acid on adrenal necrosis. Proc. Soc. Exp. Biol. Med., vol. 45, p. 482.

MORGAN, A. F., AND H. D. SIMMS 1939 Adrenal atrophy and senescence due to a vitamin deficiency. Proc. 6th Pacific Science Congress, vol. VI, p. 499.

PEARSON, P. B., V. H. MELASS AND R. M. SHERWOOD 1945 The effect of the pantothenic acid content of eggs on the amount in newly hatched chicks. Archiv. Biochem., vol. 7, p. 353.

SURE, B. 1928 Dietary requirements for fertility and lactation: A dietary sterility associated with vitamin A deficiency. J. Agric. Research, vol. 37, p. 87.

———— 1941 Dietary requirements for fertility and lactation. XXIX. The existence of a new dietary factor essential for lactation. J. Nutrition, vol. 22, p. 499.

UENO, J. 1934 Experimental study on the effects of vitamin B to female genital organs. Jap. J. Ost. and Gyn., vol. 17, p 86, 388, and 404.

WARKANY, J., AND R. C. NELSON 1942 Congenital malformations induced in rats by maternal nutritional deficiency. J. Nutrition, vol. 23, p. 321.